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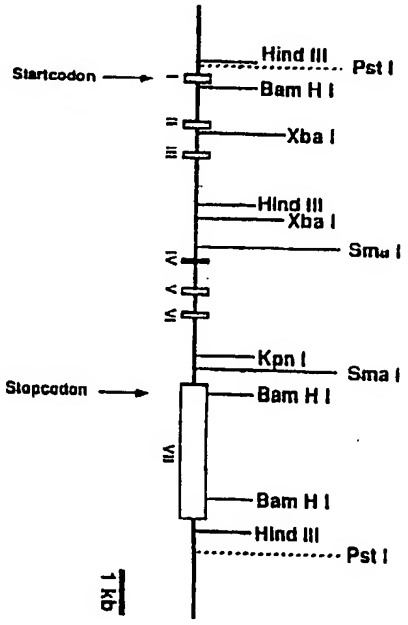

(57) Abstract

The present invention relates to nucleic acid sequences encoding amino acid sequences of the α, β, and γ subunits of the high affinity receptor for immunoglobulin E, and for amino acid sequences of the subunits. The invention further relates to a method of producing the receptor by expressing cDNA for its α, β, and γ subunits in a host cell simultaneously. Aspects of the invention are methods and compositions to produce the human beta subunit, as well as to inhibit its function, thereby treating or preventing allergic reactions.

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ISOLATION, CHARACTERIZATION, AND USE OF THE HUMAN ß SUBUNIT OF THE HIGH AFFINITY RECEPTOR FOR IMMUNOGLOBULIN E

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to DNA segments encoding the α, ß, and γ subunits of the high affinity receptor for immunoglobulin E (IgE), in particular the human ß subunit. The invention further relates to a method of producing the receptor by expressing DNA encoding its α, ß and γ subunits in a host cell simultaneously.

2. Related Art

Receptors that bind the Fc region of immunoglobulins ("Fc receptors") mediate immunoglobulin transport across membranes, stimulate a variety of cellular activities induced by antigen-antibody complexes, and possibly regulate the biosynthesis of antibodies. Three of the receptors (the receptor for polymeric immunoglobulin (Mostov et al. (1984) Nature (London) 308:37-43), the Fc receptors on macrophages and lymphocytes (Ravetch et al. (1986) Science 234:718-725), and the high affinity Fc, receptor on mast cells and basophils (Kinet et al. (1987) Biochemistry 26:4605-4610; Shimizu et al. (1988) Proc. Natl. Acad. Sci. USA 85:1907-1911; Kochan et al. (1988) Nucleic Acids Res. 16:3584) share a common feature: their immunoglobulin-binding portion contains two or more immunoglobulin-like domains.

The high affinity IgE receptor FcεRI is responsible for initiating the allergic response. Binding of allergen to receptor-bound IgE leads to cell activation and the release of mediators (such as histamine) responsible for the manifestations of allergy. This receptor is a tetrameric complex $\alpha\beta\gamma_2$ which is found on the surface of mast cells and basophils. The α and β

subunits have not been detected in other hematopoietic cells although the γ chains of FceRI are found in macrophages, NK cells and T cells where they associate with the low affinity receptor for IgG (FcγRIII) or with the T cell antigen receptor.

The genes for α and γ, both have been localized on human (Le Coniat, 1990) and mouse chromosome 1. (Huppi, 1988; Kinet et al. 1987; Kochan et al. 1988; Shimizu et al. 1988; Ra et al. 1989.) The gene for mouse β has been localized on mouse chromosome 19 and is believed to be a single gene (Huppi, 1989). The structures of the α gene in the rat (Tepler, 1989) and of the γ gene (Kuster, 1990), but not of the β gene have been characterized in the human.

The receptor with high affinity for IgE $Fc_\epsilon RI$ is found exclusively on mast cells, basophils, Langerhans cells, and related cells. Aggregation of IgE occupied $Fc_\epsilon RI$ by antigen triggers both the release of preformed mediators such as histamine and serotonin, as well as stimulation of the synthesis of leukotrienes. It is the release of these mediators that results in the allergic condition.

The most thoroughly characterized $Fc_\epsilon RI$ is that of the rat basophilic leukemia (FEL) cell line. It consists of three different subunits: (1) a 40-50 Kilodalton (Kd) glycoprotein alpha chain which contains the binding site for IgE, (2) a single 33 Kd beta chain and (3) two 7-9 Kd disulfide linked gamma chains (H. Metzger et al., Ann. Rev. Immunol. 4:419-470 (1986)).

Complementary DNA (cDNA) for the rat α subunit has been isolated (J.-P. Kinet et al., Biochemistry 26:4605-4610 (1987)). However, previously there has been no disclosure of the isolation and characterization of the β and γ subunits nor has it been possible to express IgE-binding by transfected cells (J.-P. Kinet et al., Biochemistry 26:4605-4610 (1987); A. Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911 (1988)).

Molecular cloning of some of the subunits in rodents and humans has permitted the reconstitution of surface

expressed receptor complexes by trasfection. One of the surprising findings from these studies was the differential requirement for surface expression among different species. Cotransfection of the three chains, α, β and γ is required to promote efficient surface expression of the rat (Blank, 1989) or mouse receptor (Ra, 1989). By contrast, some surface expression of the human $\alpha\gamma$ complex can be achieved by cotransfecting α and γ alone in fibroblasts, suggesting that β may not be necessary (Miller, 1989). This result and previous inability to clone the gene for the human β subunit raised the possibility that human beta might not exist and that $\alpha\gamma$ complexes might exist naturally in human cells.

The high affinity IgE receptor FcεRI is a tetrameric hetero-oligomer composed of an α chain, a β chain and two disulfide-linked γ chains (chains and subunits will be used interchangeably herein). The β chain contains four transmembrane (TM) segments and long cytoplasmic domains which are thought to play an important role in intracellular signalling. It was very difficult to determine whether a human beta subunit even existed, and if so, to isolate its gene. The present invention has overcome these difficulties and surprisingly provided cDNA clones for the human ß subunit of FcεRI.

The invention still further provides a method of producing the complete human FcεRI receptor, and for inhibiting formation of the receptor or its function, by inhibiting the ß subunit.

SUMMARY OF THE INVENTION

It is an aspect of this invention to provide nucleic acid segments encoding FcεRI subunits.

It is an aspect of this invention to provide nucleic acid sequences encoding the α, ß, and γ subunits of FcεRI. In particular, this invention relates to DNA sequences. An aspect of the present invention is the

structural characterization and the sequence of the complete human β gene and cDNA. Successful cloning of the human beta was not expected and was fraught with failures. Attempts to clone the human beta by simply using a rodent beta probe to screen various cDNA libraries failed to isolate a cDNA clone encoding human beta. Only a very short fragment (153 bp) with homology to rodent beta was isolated. However because this fragment may have been a portion of a beta-like molecule such as CD20, known to be homologous to beta in that region, PCR techniques were used to clone the human beta by using the information from the rodent beta sequence. However, although the homologies between human and rodent beta were 69% in the coding region, that was not sufficient for a PCR reaction. Human beta isolated by this method also failed.

The existence of human beta was questioned because human beta was believed not necessary for expression of the alpha-gamma complex. Studies of gene transfer indicated that the transfer by transfection of the three genes for alpha, beta and gamma was necessary for the expression of the rat and mouse receptor. However, transfection of human alpha and gamma was sufficient to promote the surface expression of the human receptor in fibroblasts suggesting that the human beta was not necessary for the surface expression of the human receptor. That result raised the interesting question of the existence of human beta.

Human beta was not necessary for the function of the alpha-gamma complex. Transfection of the cytoplasmic tail of gamma is sufficient for cell activation. Several groups made the observation that the cytoplasmic domain in the gamma chain was sufficient to mediate a number of biochemical signals leading to cell activation. These signals include tyrosine kinase activation, hydrolysis of phosphoinositides, calcium mobilization, production of IL2 in T cells, degranulation of mast cells and cell killing. It was demonstrated that the cytoplasmic domains of gamma contain a motif of 10-12 amino-acid

residues responsible for cell activation. This motif is sufficient to trigger many different signals in different cells. It is transferable, and seemed to be interchangeable. Again these findings raised the question of the existence of human beta. If the gamma chain is sufficient for cell activation, perhaps there was no need for a beta.

The inability to clone the human beta or even to detect transcripts for human beta in human cells (by using rat or mouse probes) also raised the question of the existence of human beta.

Cloning required inventive methods and persistence. The 153 bp fragment used to screen further cDNA libraries did not work. However, assuming that the 153 bp could be part of human beta even though the homology was only about 70%, a 25 kb genomic clone was found. Smaller inserts were found which seemed to hybridize specifically with oligonucleotide probes corresponding with rat beta sequences. All these inserts (a total of 11 kb) were sequenced to reconstitute the different exons in the quest for those encoding human beta. Using what should be the beginning of the first exon and the end of the coding sequence in the seventh exon from the putative human beta gene, a putative cDNA human beta sequence was generated by PCR (by using first strand reverse transcripts from human basophils as templates for the PCR reaction.)

It was demonstrated that the gene and cDNA isolated encoded human beta. The isolated gene and cDNA could correspond to a beta-like or CD20-like molecule which is homologous to rodent beta. However, the homology of 69% is not a criteria for the demonstration that these sequences encode human beta. Co-expression of alpha, beta and gamma in transfectants was preferred for the demonstration that the cDNA generated is indeed encoding human beta. However these experiments were not successful for the following reasons:

1. Co-transfection of human alpha and gamma is sufficient for surface expression and functional reconstitution of the receptor on fibroblasts.

2. When human beta cDNA is co-transfected with alpha and gamma, the efficiency of transfection is not increased.

In Fig. 20 the transfection of human alpha and gamma in COS-7 cells is shown to be sufficient for expression of the alpha-gamma complex on the surface of the transfectants. It also shows that human beta cannot replace rat beta in the expression of the rat IgE receptor. Therefore conditions were used where co-transfection of alpha and gamma does not work, to see if human beta could promote expression of the complete complex (Fig. 20). This was done by truncation of the cytoplasmic tail of human gamma. In these conditions, co-transfection of human alpha with truncated human gamma does not result in the expression of the complex. However, co-transfection of human beta (but not of CD20) with alpha and truncated gamma resulted in the expression of a functional complex capable of binding IgE. In these conditions, rat beta subunit cannot replace human beta subunit for expression of human alpha and truncated gamma subunits. This assay showed that human beta could associate specifically with the two other chains. Therefore, these new results demonstrate that human beta is a part of the human IgE receptor, and not merely a CD20-like molecule. These results reveal the previously unsuspected importance of human beta.

In Fig. 21 transfection of alpha-gamma in KU812 cells showed very little expression of receptors. The level of expression is similar to the level obtained after transfection of beta and gamma. Therefore this level may be attributable to the endogenous alpha (for beta and gamma transfection) or to the endogenous beta (for alpha and gamma transfection). By contrast the level of expression after co-transfection of the three cDNAs is very substantial.

The conclusions are:

1. In mast cells and basophils, what regulates the level of expression of the receptor may be different than in fibroblasts.

2. In human mast cells and basophils, receptor expression requires the presence of alpha, beta gene and gamma, whereas in transfected fibroblasts, human alpha and gamma are sufficient.

The human beta subunit gene spans approximately 10 kb and contains seven exons. There is a single transcription initiation site preceded by a TATA box. The first exon codes for the 5' untranslated region and a portion of the N-terminal cytoplasmic tail. Transmembrane (TM) 1 is encoded in exon 2 and 3, TM 2 in exon 3 and 4, TM 3 in exon 5 and TM 4 in exon 6. The seventh and final exon encodes the end of the C-terminal cytoplasmic tail and the 3' untranslated sequence. The human β gene appears to be a single copy gene.

Two corresponding transcripts, detected as a doublet around 3.9 kb, are present in cells of mast cell and basophil lineage from different individuals but not in the other hematopoietic cells tested. The human β protein is homologous to rodent β. The consensus amino acid sequences for human, mouse and rat β show 69% identical residues.

It is a further aspect of the invention to provide polypeptides corresponding to the α, β, and γ subunits of FcεRI, more particularly to the human ß subunit isolated from its natural environment. This may be defined to include the amino acid sequences of the polypeptides either produced by recombinant methods, or synthesized by apparatus known to those of skill in the art, or isolated and purified by protein isolation and purification methods. The polypeptides comprise the entire amino acid sequence, or selected portions thereof, for example, portions (domains) of the human beta subunit that are essential for (1) assembly of the receptor; (2) cell activation, and/or (3) complexing with the alpha and gamma subunits. "Natural environment" may be defined to include the subunits in the cells in which they naturally

occur, in the form and with other types of proteins and cellular components generally in structural or functional association with the subunits.

It is another aspect of the invention to provide a recombinant DNA molecule comprising a vector and a DNA segment encoding the α, ß, or γ subunits of FcεRI.

It is a further aspect of the invention to provide a cell that contains the above-described recombinant DNA molecule.

It is another object of the invention to provide a method of producing polypeptides having amino acid sequences corresponding to the α, ß, and γ subunits of FcεRI, both in rodent and human species.

Analysis of the surface expression of transfected receptors in fibroblast-like cells indicates that human αγ and αβγ complexes are expressed with comparable efficiency. Unexpectedly, assembly rules were different in other human cells. In addition, human β interacts with human α more efficiently than does rat β. By contrast, both rat and mouse β interact with their corresponding α chains much more efficiently than does human β, demonstrating a strong species specificity of the α-β interaction.

It is a further object of the invention to provide a method of producing a functional FcεRI receptor.

In one embodiment, the present invention relates to DNA segments that code for polypeptides having amino acid sequences corresponding to the α, ß, and γ subunits of FcεRI.

In another embodiment, the present invention relates to polypeptides having amino acid sequences corresponding to the α, ß, and γ subunits of FcεRI.

In a further embodiment, the present invention relates to recombinant DNA molecules comprising a vector and a DNA segment that codes for a polypeptide having an amino acid sequence corresponding to the α, ß or γ subunits of FcεRI.

In yet another embodiment, the present invention relates to a cell that contains the above-described recombinant DNA molecule.

In a further embodiment, the present invention relates to a method of producing polypeptides having amino acid sequences corresponding to the α, ß, and γ subunits of FCERI.

In another embodiment, the present invention relates to a method of producing a functional FCERI receptor comprising introducing into a host cell DNA segments encoding the α, ß, and γ subunits of FcεRI; and effecting expression of said DNA segments under conditions such that said receptor is formed. Expression of the receptor on the surface of cells COS-7 or CHO is achieved by the present invention when the cDNA for all three subunits of FCERI are simultaneously cotransfected. This success in expression of IgE binding permits detailed analysis of the IgE-receptor interaction and thus enables the development of therapeutically effective inhibitors.

An aspect of the invention is to stem the cascade of allergic responses resulting from aggregation of the high affinity receptor for IgE, by inhibiting the essential participation of the human beta subunit. The beta subunit is the target to inhibit receptor aggregation and/or the function of the translate signal. Such an inhibition has widespread applications for prevention and treatment of allergic diseases because the undesirable events cascading from the receptor-IgE interaction are allergen independent and arise from various cell types: mast cells. basophils, Langerhans cells and the like.

Inhibitors of beta include chemical preparations that attack the structure or function of the chain, anti-sense nucleic acid sequences, amino acid sequences capable of binding to the beta subunit polypeptide, and monoclonal antibodies directed to the subunit.

Effective amounts of the beta subunit inhibitors will be determined after *in vitro* cell assays, assays in animal models, and clinical trials.

Effective amounts of the inhibitors will be combined with a pharmaceutically acceptable carrier. Because of the variety of cell types in which the allergic response is related to the FcεRI, and because the reaction is allergen independent, route of administration may be either systemic or atopic.

Candidate inhibitor substances are tested by methods disclosed herein.

In vitro assays for inhibitor substances are provided through host cells transfected with nucleic acid sequences for encoding the human alpha, beta and gamma subunits, complexed or incubated with inhibitors. Cell activation effected by the FcεRI receptor is triggered and compared in the presence versus absence of inhibitors. Many assays are available.

Further objects and advantages of the present invention will be clear from the following description and examples.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. The nucleotide sequence (SEQ ID NO:10) and predicted amino acid sequence (SEQ ID NO:11) of human FcεRI alpha cDNA are shown.

FIG. 2. The amino acid sequence homology of rat FcεRI alpha subunit (R) (SEQ ID NO:12), human FcεRI alpha subunit (A) (SEQ ID NO:13), and mouse FcεRI alpha subunit (M) (SEQ ID NO:14) are shown. The regions of identity between the three are boxed. The number one position corresponds to the site of the predicted mature N-terminus of each protein.

FIG. 3. A flow chart showing the construction of eukaryotic expression vectors which direct the synthesis of a complete biologically active FcεRI alpha chain (pHAI, pHAII) or a soluble, secreted, biologically active FcεRI alpha chain (pHASI, pHASII) is presented. The sequence shown in this Figure is also disclosed in SEQ ID NO:20.

FIG. 4. A flow chart showing the construction of a prokaryotic expression vector which directs the synthesis of a soluble biologically active FcεRI alpha chain (which consists of amino acid residues 26-204) is presented. The sequences shown in the pEVA construct are also shown in SEQ ID NOs. 15-17; the sequences shown in the pEVHA construct are also shown in SEQ ID NOs. 18 and 19; the sequences shown in the pEVHAS construct are also shown in SEQ ID NOs. 20 and 21.

FIG. 5. Restriction maps for β cDNAs and strategy by which they were sequenced. The open rectangle indicates the sequence predicted to code for the β subunit; the lines indicate the 5' and 3' untranslated regions. The upper scheme shows the 1.5 kilobase (kb) clone containing a Pst I cleavage site. The lower scheme shows a 2.4-kb clone containing a ClaI cleavage site. The 3' region of the latter has been truncated as indicated by the slashes. Its untranslated portion was sequenced as completely as the rest of the clone. Restriction sites are indicated by vertical bars: Hf, HinfI; *Hh*, *Hha I*; Al, *Alu I*; Hp, *HphI*; Av, *Ava II*; Ac, *Acc I*; Ec, *EcoRI*; Hd, *HindIII*. The horizontal arrows show the direction and extent of sequencing by the dideoxynucleotide chain-termination method.

FIG. 6. (A) Nucleotide (SEQ ID NO:22) and deduced amino acid (SEQ ID NO:23) sequences of the cDNA coding for the β subunit. Beginning at the arrowhead (▾), an alternative sequence (B) was observed in six clones. The putative transmembrane domains are underlined. The tryptic peptides of the β subunit, from which the amino acid sequences were determined directly, are bracked (<>). A putative poly (A) signal near the end is underlined. (B) Continuation of the nucleotide sequence (SEQ ID NO:24) of the deleted form of β cDNA, 3' to the junction indicated in A (▾).

FIG. 7. Expression of cDNA coding for the β subunit. (A) Comparison of *in vivo* and *in vitro* translation products. RBL cells were grown in [^{35}S]cysteine containing medium. The detergent extract of the cells was

precipitated with mAbß(JRK) and, after vigorous washing, extracted with sample buffer and electrophoresed (lane 1). This experiment employed concentrations of detergent high enough to dissociate the receptor completely. A transcript from the ß cDNA was treated *in vitro* in [^{35}S]methionine-containing medium (lanes 2, 3, and 5).

A control incubation contained no cDNA (lane 4). The mixtures were allowed to react with monoclonal antibodies to the ß subunit after a clearing immunoprecipitation. The specific washed precipitates were dissolved in sample buffer and electrophoresed: lanes 2 and 4, mAbß(JRK); lane 3, mAbfl(NB); lane 5, irrelevant monoclonal antibody [mAbß(LB)]. An autoradiograph of the 12.5% polyacrylamide gel on which the specimens were analyzed under reducing conditions is shown. (B) Localization of one epitome to the NH_2-terminal peptide of the ß subunit. A ß cDNA-containing vector was digested with HhaI before transcription using T7 polymerase. The resulting mRNA was translated to generate an NH_2-terminal peptide of the ß subunit ß(amino acid 1-21) labeled with [^{35}S]methionine. The mixture was allowed to react with mAbß(JRK) (lane 1) and the irrelevant mAb(LB) (lane 2). The precipitates were analyzed on a 17% gel under nonreducing conditions. (C) Expression by *E. coli* of a COOH-terminal fragment of the ß subunit. A HinfI fragment, containing nucleotides 499-787, was subcloned into an *E. coli* expression vector (Crowl et al. (1985) Gene 38:31-38) and extracts were prepared. The proteins were electrophoresed as in A and transferred to nitrocellulose paper. The latter was allowed to react sequentially with monoclonal antibody mAbß(NB), developed with alkaline phosphatase-conjugated goat anti-mouse IgG (Fc), and developed in the usual way (Rivera et al. (1988) Mol. Immunol.). An enlargement of the lower half of the immunoblot is shown. Lane 1, extract from transformant without insert; lane 2, extract from transformant with insert in wrong direction; lane 3, extract from transformant with insert correctly oriented. (D) Reactivity of ß subunits with polyclonal antibodies induced by *E. coli*-expressed HinfI fragments. Purified

IgE-receptor complexes were electrophoresed, transferred to nitrocellulose paper, and allowed to react with antibodies and subsequently with an appropriate alkaline phosphatase-conjugated anti-immunoglobulin antibody. Lane 1, mAbß(JRK); lane 2, mAbß(NB); lane 3, immune serum to fragment A; lane 5, immune serum to fragment B; lanes 4 and 6, preimmune sera corresponding to the immune sera in lanes 3 and 5, respectively; lanes 7 and 8, second antibody only. This gel was run without molecular weight standards.

FIG. 8. Hydropathicity plot of predicted sequence for the ß subunit. The procedure and hydropathicity scale recommended by Engleman et al. (Engelman et al. (1986) Annu. Rev. Biophys. Biophys. Chem. 15:321-353) was used. The net hydropathicity value for the 20 amino acids for each successive "window" is plotted at the position corresponding to the 10th residue. A net free energy of >20 kcal (1 cal=4.18 J) for transfer to water suggests a transmembrane segment (Engelman et al. (1986) Annu. Rev. Biophys. Biophys. Chem. 15:321-353).

FIG. 9. Nucleotide sequence (SEQ ID NO:26) of the γ subunit of rat FcεRI and the amino acid sequence (SEQ ID NO:27) that it predicts. The putative transmembrane domain is underlined. Amino acid resides are numbered starting with the first residue of the mature protein. Residues 5' to residue 1 have negative numbers and include the residues encoding a putative signal peptide according to the criteria of G. von Heijne (Nucleic Acids Res. 14:4683-4690 (1986)). The N-terminal and C-terminal cleavage sites are indicated by an arrow. The four tryptic peptides which were covered and sequenced are bracketed. An asterisk denotes an ambiguous residue in the sequence of the first tryptic peptide.

FIG. 10. Hydropathicity plot of predicted sequences of FcεRI α subunit (panel A), ß subunit (panel B) and γ subunit (panel C). The hydropathicity scale is according to Engelman et al. (Ann. Rev. Biophys. Biophys. Chem. 15:321-353 (1986)). The summed hydropathicity values for

the 20 amino acids in successive "windows" is plotted at the position corresponding to the tenth residue.

FIG. 11. Formation of IgE rosettes by transfected COS 7 cells and RBL cells. COS 7 cells were cotransfected with the coding portions of α, ß and γ cDNAs and sensitized with mouse IgE anti-DNP before being exposed to red cells derivatized with TNP (Panel A). As a positive control, RBL cells were similarly tested for rosette formation (Panel C). The specificity of the rosetting assay was assessed by preincubating the cotransfected COS 7 cells (Panel B) and RBL cells (Panel D) with rat IgE (which lacks the anti-DNP activity) prior to the addition of the mouse anti-DNP IgE.

FIG. 12. Model of the tetrameric high affinity receptor for IgE. The polypeptides (SEQ ID NOs. 28-30) are shown in their fully processed form. The receptor is oriented such that the large extracellular portion of the α subunit is shown at the top and the remainder of the chain on the left. To the right of the α subunit (SEQ ID NO:28) is the ß subunit (SEQ ID NO:29) with its four transmembrane segments and to the right of it, the dimer of γ chains (SEQ ID NO:30). Cysteines 26 and 68 and cysteines 107 and 151 in the α chain are paired as they are likely to be disulfide linked, as are the homologous cysteines in the Fcγ receptors (M. Hibbs et al, J. Immunol. 140:544-550 (1988)). The putative transmembrane segments have all been shown as consisting of 21 residues and would be expected to be in an α-helical conformation. The single letter code for amino acids is used (M. Dayhoff et al., in Atlas of Protein Sequence and Structure, Suppl. 3, ed. M. Dayhoff, 363-373, Natl. Biomed. Res. Fndtn., Washington D.C. (1978)). Every 10th residue (starting from the N-terminus) is shaded.

FIG. 13. Restriction map structure of the human ß gene and exon-intron are shown. The positions of the 7 exons are depicted by boxes. The location of the start and stop codon is indicated.

FIG. 14. Nucleotide sequence (SEQ ID NO:31) of the human FcεRI β chain gene. The 7 exons are shown in bold.

The numbering of nucleotides is relative to the start codon. The TATAA box, translation initiation codon (ATG), termination codon (TAA) and the potential polyadenylation signals (AATAAA) are underlined. Bases which not determined with certainty are denoted as N.

FIG. 15. Comparison of the human β gene and rat β cDNA sequences by a dot matrix blot. The Pustell DNA Matrix of the Macvector program was used with a window of 30 nucleotides and a minimum score of 63%. The Roman numerals indicated on the left correspond to the seven exons.

FIG. 16. Presence of transcripts in basophils are shown. Ten micrograms of total RNA from basophil enriched leukocytes and various other cells were fractionated on a denaturing agarose gel before being transferred to Nytran membranes and hybridized with human β cDNA probes (nucleotides +306 to +456 for Panel A and nucleotides -2 to + 790 in Panel C). The membrane shown in Panel A was stripped and rehybridized with a full length human α cDNA probe (Panel B).

FIG. 17. Determination of the transcription initiation site.

Panel A: RNA from basophils was reverse transcribed, poly A+ tailed at both ends with terminal transferase and amplified with PCR. The amplified product (cDNA) and the genomic DNA (gene) were sequenced with an identical primer and the respective sequencing reactions were run in parallel on a 8% acrylamide gel. The arrow marks the transcription start site. Panel B: RNA from basophils (lane 1) or tRNA (lane 2) were used in the primer extension and the extended products analyzed on a 5% polyacrylamide urea gel in parallel with the sequencing reactions of the genomic DNA. The arrow marks the transcription start site.

FIG. 18. Southern blot analysis of genomic DNA obtained from five different individuals. The DNAs were subjected to distinct restriction endonuclease digestions, blotted and hybridized with the human full length cDNA for the beta subunit. The numbers on the top

indicate the different individuals while each panel corresponds to a different restriction digest. Size standards are indicated on the right.

FIG. 19. Amino acid sequence of the FcεRI human β subunit (SEQ ID NO:32) and alignment with rat (SEQ ID NO:33) and mouse (SEQ ID NO:34) β. Identical and non-identical amino acid residues are indicated by capital and lower case letters respectively. The identities and closely related exchanges are marked ^ in the query line while the distantly related exchanges are denoted by a dot. Non-homologous exchanges show no marking in the query line. The gaps are indicated by a hyphen. The transmembrane domains are underlined and the splice sites indicated with vertical bars.

FIG. 20. Results of FACS analysis showing IgE binding in COS-7 cells transfected with various combinations of FcεRI subunits.

FIG. 21. Results of FACS analysis showing IgE binding in cells of a basophil line (KU812) transfected with various combinations of FcεRI subunits.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates, in part, to DNA sequences which code for polypeptides corresponding to the subunits of human FcεRI.

More specifically, the present invention relates to DNA segments (for example, cDNA molecules) coding for polypeptides having amino acid sequences corresponding to the α,β and γ subunits of FcεRI. In one embodiment, the DNA segments have the sequence shown in FIG. 1, 6, 9, or 14 (SEQ ID NOs. 10, 22 and 24, 26 and 31), allelic or species variation thereof, or a unique portion of such a sequence (unique portion being defined herein as at least 15-18 bases). In another embodiment, the DNA segments encode the amino acid sequence shown in FIG. 1 (SEQ ID NO:11), 6 (SEQ ID NOs. 23 and 24), 9 (SEQ ID NO:27), or 19 (SEQ ID NOs. 32-34), or allelic or species variation

thereof, or a unique portion of such a sequence (unique portion being defined herein as at least 5-6 amino acids).

Allelic or species variations are defined as substitutions, deletions, or other alterations in the nucleotide or amino acid sequence that do not eliminate the function of the subunits as defined herein. For some uses, the nucleotide sequence may be deliberately altered to, e.g., test the effects of such alteration on the function of the beta subunit, or to produce subunits which are inactiviated for certain purposes.

In another embodiment, the present invention relates to polypeptides having amino acid sequences corresponding to the α,β and γ subunits of FcεRI. In one preferred embodiment, the polypeptides have amino acid sequences as shown in FIG. 1, 6, 9, and 19 (SEQ ID NOs. 11, 23 and 24, 27 and 32-34, respectively) or allelic or species variations thereof, or a unique portion of such sequences (unique portion being defined herein as at least 5-6 amino acids).

In another embodiment, the present invention relates to a recombinant DNA molecule comprising a vector (for example - plasmid or viral vector) and a DNA segment coding for a polypeptide corresponding to the α,β or γ subunit of FcεRI, as described above. In a preferred embodiment, the encoding segment is present in the vector operably linked to a promoter.

In a further embodiment, the present invention relates to a cell containing the above described recombinant DNA molecule. Suitable host cells include procaryotes (such as bacteria, including E. coli) and both lower eucaryotes (for example yeast) and higher eucaryotes (for example, mammalian cells). Introduction of the recombinant molecule into the host cell can be effected using methods known in the art.

In another embodiment, the present invention relates to a method of producing the above described polypeptides, comprising culturing the above described

host cells under conditions such that said polypeptide is produced, and isolating said polypeptide.

In a further embodiment, the present invention relates to a method of producing a functional FcεRI receptor comprising introducing into a host cell DNA segments encoding the α,β and γ subunits of FcεRI and effecting expression of said segments under conditions such that said receptor is formed.

The nucleic acid sequences and polypeptides according to this invention exhibit a number of utilities including but not limited to:

1. Utilizing the polypeptide or a fragment thereof as an antagonist to prevent allergic response, or as a reagent in a drug screening assay.

2. Utilizing the polypeptide as a therapeutic agent.

3. Utilizing the polypeptide for monitoring IgE levels in patients.

4. Utilizing the nucleic acid sequence to synthesize polypeptides which will be used for the above purposes.

5. Utilizing the nucleic acid sequences to synthesize cDNA sequences to construct DNA useful in diagnostic assays.

The present invention will be illustrated in further detail in the following examples. These examples are included for illustrative purposes and should not be considered to limit the present invention.

EXAMPLE 1

Isolation of cDNA Clones for the Alpha Subunit of Human FcεRI

RNA was extracted from FUBl2 cells as described by Kishi, Leukemia Research, 9,381 (1985) by the guanidium isothiocyanate procedure of Chirgwin, et al., Biochemistry, 18,5294 (1979) and poly(A) mRNA was isolated by oligo-dt chromatography according to the methods of Aviv, et al., P.N.A.S. U.S.A., 69,1408 (1972).

cDNA synthesis was performed as previously described Kinet, et al., Biochemistry, 26,2569 (1987). The resulting cDNA molecules were ligated to EcoRI linkers, digested with the restriction enzyme EcoRI, size fractioned and ligated to λgt11 EcoRI arms as set forth in Young et al., Science 222,778 (1983). The cDNA insert containing λgt11 DNA was packaged into bacteriophage lambda particles and amplified on Y1090. A total of 1.2x106 independent cDNA clones were obtained. The cDNA library was plated onto Y1090 on 150 mm_2 plates (10^5 per plate) and transferred to nitrocellulase filters. The cDNA library filters were screened by in situ hybridization using a nick translated cDNA fragment as in Kochan, et al., Cell, 44,689 (1986). The cDNA fragment was obtained from the rat FcεRI alpha cDNA corresponding to nucleotides 119-781. Positive plaques were identified, purified and the cDNA inserts were subcloned, using standard techniques, into the pGEM vectors (Promega Biotech, Madison, Wisconsin). The cDNA insert was mapped by restriction enzyme analysis, subcloned into derivatives of pGEM and sequenced using the dideoxynucleotide method of Sanger et al., P.N.A.S., 74,5463 (1977) following the GemSeq double strand DNA sequencing system protocol from Promega Biotech (Madison, Wisconsin). The DNA sequence was determined for both strands of the cDNA clone pLJ663 (nucleotides 1-1151) and for 300 bp of each end of clone pLJ 587 (nucleotides 658-1198). No discrepancy in DNA sequence between the two cDNA clones was observed.

The sequence for the human FcεRI alpha cDNA is presented in FIG. 1 and SEQ ID NO:10. The predicted amino acid sequence for the human FcεRI alpha polypeptide is shown below the nucleotide sequence and in SEQ ID NO:11, beginning with methionine at nucleotide 107-109 and ending with asparagine at nucleotide 875-877. The site of the predicted mature N-terminus was determined to be valine at nucleotide 182-184 according to the rules set forth by von Heijne, Eur. Journal of Biochem; 137,17; and Nucleic Acid Research, 14,4683 (1986).

These results predict a 25 amino acid signal peptide. The rest of the cDNA sequence suggests that the human Fc$_\epsilon$RI alpha chain contains about 179-224 residues with 2 homologous domains (14 out of 25 residues are identical; residues 80-104 and 163-190), a 20-residue transmembrane segment (residues 205-224) and a 33 residue cytoplasmic domain containing 8 basic amino acids. Overall, there is 47% identity between the human and rat Fc$_\epsilon$RI alpha sequences, and 46% identity between the human FRI alpha and mouse FcGR alpha (FIG. 2 and SEQ ID NOs. 12-14). The greatest level of homology is within the transmembrane region where 9 amino acids surrounding the common aspartic acid residue are identical.

EXAMPLE 2

Expression of the Human Fc$_\epsilon$RI Alpha Complete and Soluble Forms in Eukaryotic Cells

Using the recombinant cDNA clone for the human Fc$_\epsilon$RI alpha chain, it is possible to introduce these coding sequences into an appropriate eukaryotic expression vector to direct the synthesis of large amounts of both a complete and soluble form of the alpha chain. For surface expression it may necessary that the alpha subunit be complexed with the beta or gamma subunit whereas for the eukaryotic expression of the secreted form of the alpha subunit this may not necessary. An appropriate vector for the purpose is pBC12BI which has previously been described in Cullen, (1987) Methods in Enzymology 152, Academic Press, 684. Construction of expression vectors coding for the complete alpha chain can be isolated as follows (FIG. 3): A unique BglII-SspI fragment (nucleotides 65-898) is isolated from pLJ663, the BglII end is filled in with DNA polymerase I Klenow fragment and ligated into pBC12BI which has been restricted with either HindIII-BamHI or HindIII-SmaI (the ends are made blunt by filling in with DNA polymerase I Klenow fragment). The reason for attempting two different constructions is that the former contains

a 3' intron while the latter does not. The presence or absence of introns may affect the levels of the alpha protein which are synthesized in cells transfected by these vectors. Construction of expression vectors coding for the soluble form of the alpha chain would be accomplished by introducing a termination codon at nucleotides 719-721 of the coding region in the alpha chain of the expression vectors noted above (pHAI, pHAII, FIG. 3). This would remove the putative transmembrane and cytoplasmic regions resulting in the synthesis of a secreted soluble form of the human alpha chain. Introduction of a termination codon is accomplished by oligonucleotide-directed site specific mutagenesis as outlined by Morinaga et al., Bio. Tech., 2, 636 (1984). The sequence of the oligonucleotide will be 5' AAGTACTGGCTATGATTTTTTATCCCATTG 3' (SEQ ID NO:1). The resulting expression vectors are pHASI and pHASII (FIG. 3) and these will direct the synthesis of a truncated alpha protein corresponding to amino acids 1-204. Expression of this protein in eukaryotic cells will result in synthesis of a mature, IgE binding protein encompassing amino acid residues 26-204.

The expression vectors are then introduced into suitable eukaryotic cells such as CHO or COS by standard techniques such as those set forth in Cullen, (1987), Methods in Enzymology, Academic Press, NY 152:684, in the presence of a selectable marker such as G418 or Methotrexate resistance. The selectable marker for Methotrexate resistance has an added advantage, since the levels of expression can be amplified by introducing the cells to higher levels of drugs. The synthesis of protein is monitored by demonstrating the ability of human IgE (or rat IgE) to bind to these cells (in the case of the complete alpha chain), or in the case of the soluble form of the alpha chain, to demonstrate that the protein secreted from these cells has the ability to bind IgE in the presence or absence of the beta.

EXAMPLE 3

Expression of the Human FcεRI Alpha Soluble Form in Prokaryotic Cells

Using the recombinant cDNA clone for the human FcεRI alpha chain, it is possible to introduce these coding sequences into an appropriate prokaryotic expression vector to direct the synthesis of large amounts of a soluble (non-membrane bound) IgE binding polypeptide derived from the alpha chain. An appropriate vector for this purpose is pEV-1 which has been described by Crowl, et al., Gene, 38, 31 (1985). Construction of an expression vector coding for a soluble alpha chain can be isolated as set forth in FIG. 4: a unique MstII-SspI fragment (nucleotides 195-898 is isolated from pLJ663, the MstII end is filled in with DNA polymerase I Klenow fragment and ligated into pEV-1 which has been restricted with EcoRI, and the ends filled in with Klenow (FIG. 4, pEVA). The N-terminus of the mature alpha chain is reconstructed by oligonucleotide directed-site specific mutagenesis. The sequence of the oligonucleotide will be -5' GAATTAATATGGTCCCTCAGAAACCTAAGGTCTCCTTG 3' (SEQ ID NO:2). Introduction of this sequence into the expression vector pEVA aligns the Methionine residue of the EV-1 vector next to Valine-26 (the predicted mature N-terminus of the alpha chain) followed by amino acid residues 27-204 (pEVHA, FIG. 4). Reconstruction of the soluble form FcεRI alpha is accomplished by oligonucleotide site-directed mutagenesis. The sequence of the oligonucleotide will be 5' - AAGTACTGGCTATGATTTTTTATCCCATTG - 3' (SEQ ID NO:3). Introduction of this sequence into the expression vector, terminates polypeptide synthesis just prior to the start of the transmembrane region. The protein thus encoded by expression vector pEVHAS, should faithfully direct the synthesis of a soluble form of the alpha chain, corresponding to amino acid residues 26-204. This expression vector is then transformed into suitable hosts.

EXAMPLE 4

Isolation and Sequence Analysis of Peptides of the Beta Subunit of FcεRI

Since repeated attempts to sequence intact ß chains were unsuccessful, peptides were isolated from tryptic digests. Electroeluted ß subunits from polyacrylamide gels were prepared as described (Alcaraz et al. (1987) Biochemistry 26:2569-2575). Tryptic peptides were separated by high-pressure liquid chromatography and sequenced as before (Kinet et al. (1987) Biochemistry 26:4605-4610). A peptide (no. 1) isolated from an initial digest had the sequence (SEQ ID NO:4) Tyr-Glu-Glu-Leu-His-Val-Tyr-Ser-Pro-Ile-Tyr-Ser-Ala-Leu-Glu-Asp-Thr. The same peptide from later digests showed an additional leucine at the NH_2 terminus and an arginine at the COOH terminus. The sequences of three other peptides, each isolated in substantial yields, are indicated in a subsequent figure.

EXAMPLE 5

Cloning and Sequencing of cDNA clones of the Beta Subunit of FcεRI

RNA extracted from rat basophilic leukemia (RBL) cells by the guanidinium isothiocyanate method (Chirgwin et al. (1979) Biochemistry 18:5294-5299) was fractionated on an oligo(dT)- cellulose column (Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab., Cold Spring Harbor, NY) and used to construct a pUC-9 and a λgt11 library (Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab., Cold Spring Harbor, NY; Young and Davies (1983) Proc. Natl. Acad. Sci. USA 80:1194-1198). The initial sequence obtained for peptide 1 was used to construct two 26-mer oligonucleotides of 32-fold degeneracy: 5' -GGIGA(A/G) TA(G/C) ACATGIA(A/G) (C/T) TC (C/T) TCATA-3' (SEQ ID NO:5) and 5'-GGICT(A/G)TA(G/C)ACATGIA(A/G) (C/T)TC(C/T)TCATA 3' (SEQ ID NO:6). A λgt11 library constructed from mRNA of RBL

cells was screened with 1:1 mixture of these 15 oligonucleotides. Colonies were screened as in Kinet et al. (1987) Biochemistry 26:4605-4610, using oligonucleotides prepared on a model 380A automated DNA synthesizer (Applied Biosystems, Foster City, CA). Six positive clones gave similar restriction patterns. cDNA inserts were subcloned into pGEM-4 or pGEM-3Z and the resulting double-stranded DNA was sequenced with the GemseqRT sequencing system according to the method recommended by the supplier (Promega Biotec, Madison, WI). Twenty-mer oligonucleotides, corresponding to previously sequenced regions by this method, were used as lprimers to generate overlapping sequences otherwise difficult to obtain. In some instances, DNA sequencing was performed using Sequenase as recommended by the supplier (United States Biochemical, Cleveland). The clone containing the longest insert was sequenced according to the strategy shown in the upper portion of FIG. 5. The sequence predicts possible starting codons at nucleotides 46-48 and 55-57, which would yield a polypeptide of 246 or 243 residues, respectively (FIG. 6A and SEQ ID NO:22). The predicted M_r of about 27,000 is some 20% less than the apparent molecular weight of ß subunits when analyzed on polyacrylamide gels (Holowka and Metzger (1982) Mol. Immunol. 19:219-227). In addition, no in-frame stop codon was apparent upstream of the start codon. To rule out the possibility that the true start codon was still further 5', the cDNA library was rescreened with a restriction fragment (nucleotides 7-474) and with a synthetic oligonucleotide probe (nucleotides 3-32). Twenty-eight additional clones were isolated and their restriction patterns were examined. Twenty were similar to the original clones. Only six additional nucleotides at the 5' end (nucleotides 1-6, FIG. 6A were identified. Early termination was found in six clones, which otherwise had the same sequence through nucleotide 375 (FIG. 6B and SEQ ID NO:24). One 2.4-kb clone had cytidine 473 substituted with an adenine. This substitution abolishes the Pst I site and creates a new

Cla I site at nucleotide 470. Also thereby, Ala-140 would become Asp-140 (FIG. 6A).

Finally, one clone extended ≈350 base pairs (bp) in the 5' direction. The junction with the sequence shown in FIG. 6A was (SEQ ID NO:7) AATAAAACAAAAAAAAAAAATG, the last two nucleotides of the newly generated ATG corresponding to nucleotides 8 and 9 of the previous sequence. It is likely that this clone simply resulted from the ligation of two independent cDNAs. Screening of the pUC-9 library revealed three clones. However, the sequence of none of these extended 5' beyond nucleotide 84.

EXAMPLE 6
RNA Transfer Blotting

RNA transfer blotting was performed under high stringency using a *Pst I* fragment probe (nucleotides 1-474). Thirty micrograms of total RNA was run on a 1% agarose gel containing 2% formaldehyde and blotted to nitrocellulose filters (Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab., Cold Spring Harbor, NY). The filters were hybridized with a restriction fragment of the B cDNA (nucleotides 1-474) as described (Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab., Cold Spring Harbor, NY) and washed with 15 mM NaCl/1.5 mM sodium citrate at 65°C. RBL cells yielded two major bands at ≈2.7kb and 1.75kb with the upper band having about twice the intensity of the lower one. A minor band 1.2kb was also noted. Negative results were obtained with a variety of cells that do not express high-affinity IgE receptors: the rat pituitary line GH3 (American Type Culture Collection no. CCL82.1), the rat glial cell line C6 (no. CCL107), the mouse Leydig cell line 1-10 (no. CCL83), and, notably, the mouse monocytic line J774 (no. T1B67) and the rat lymphoma "NTD" (Rivera et al. (1988) Mol. Immunol.)

EXAMPLE 7

In vitro transcription and translation

cDNAs corresponding to the ß subunit and various mutated or truncated forms thereof were subcloned into either pGEM-4 or pGEM-3Z transcript ion vectors (Promega Biotec). The ß clone containing the *Pst I* site was transcribed *in vitro* with T7 RNA polymerase. Unlabeled RNAs were synthesized using either SP6 or T7 polymerase as recommended by the supplier. Capping reactions were performed as reported (Contreras et al. (1982) Nucleic Acids Res. 10: 6353-6362). After digestion of the template with RNase-free DNase I, the RNAs were purified further by extraction with phenol/chloroform and three precipitations from ethanol. The RNA was then translated with a micrococcal nuclease-treated lysate of rabbit reticulocytes in the presence of [S^{35}] methionine as recommended by the supplier (Promega Biotec). The products of translation were diluted 1:1 with 20 mM detergent {3-[3-(cholamidopropyl)dimethylammonio]-1-propane sulfonate in borate-buffered saline (pH 8) containing 30 µl of aprotinin per ml, 175 µg of phenylmethyl-sulfonyl fluoride per ml, 10 µg of leupeptin per ml, and 5 µg of pepstatin per ml and immunoprecipitated with monoclonal antibodies as described (Rivera et al. (1988) Mol. Immunol.). The unfractionated translated material showed a major component at M_r 32,000 compared to the control from which the RNA had been omitted or an alternative RNA (brome mosaic virus) had been substituted.

The isolation of antibodies was as follows: *Escherichia coli* transformed with an expression vector containing the desired restriction fragments (Crowl et al. (1985) Gene 38:31-38; Portnoy et al. (1986) J. Biol. Chem. 261:14697-14703) were cultured and induced, and the fraction enriched for the recombinant protein was prepared as described (Portnoy et al. (1986) J. Biol. Chem. 261: 14697-14703). After separation on polyacrylamide gels in sodium dodecyl sulfate ($NaDodSO_4$)

the transformant-specific protein was eluted and used to immunize rabbits. Approximately 100 μg of protein was injected in complete Freund's adjuvant; this was followed by a booster injection of 25 μg of protein in incomplete adjuvant. The isolation and characterization of monoclonal anti-ß antibodies mAbß(JRX) and mAbß(NB) (the latter, was obtained from David Halowka, Cornell University) have been described (Rivera et al. (1988) Mol. Immunol. 25:647-661).

The monoclonal anti-ß antibodies mAbß(JRk) and mAbß(NB) (Rivera et al. (1988) Mol. Immunol.) (FIG. 7A, lanes 2 and 3) - but not an irrelevant antibody (lane 5)- precipitated radioactive material, which on polyacrylamide gels in $NaDodSO_4$ showed a major band at M_r 32000. This band had the identical mobility as the upper band of the doublet precipitated by mAbß(JRK) from an extract of labeled RBL cells (lane 1). Although not seen well in the reproduction, the autoradiogram showed that the material synthesized *in vitro* also contained the lower molecular weight component seen the *in vivo* synthesized 0 chains. The mobility of the *in vitro* synthesized protein was unaltered by reduction as has been previously observed with the ß subunit. The clone containing the *Cla I* site (which lacks the first ATG codon) led to the synthesis of a protein whose mobility on gels was indistinguishable from that for the clone containing the *Pst I* site. On the other hand, an aberrant clone containing the newly generated ATG (above) induced the synthesis of a somewhat larger protein with an apparent M_r of 33,500. *In vitro* translation of a transcript coding for the NH_2-terminal 21 amino acids of the ß subunit led to a product precipitable by mAbß(JRK) (FIG. 7B).

EXAMPLE 8

Expression of the Beta Subunit of FcεRI in *E. Coli*

Two HinfI fragments (A, nucleotides 106-498; B, nucleotides 499-787) were individually subcloned into an

E. coli expression vector, and extracts were prepared from the induced cultures. The results of one immunoblotting experiment are shown in FIG. 7C. The material extracted from the bacteria transformed with a vector containing the HinfI fragment B exhibited a M_r 14,000 component reactive with mAbß(NB) but not with mAbß(JRK) (FIG. 7C, lane 3). The extract from the transformants containing the more NH_2-terminal HinfI fragment A (residues 17-148) reacted with neither antibody (compare with above). Rabbit antibodies generated by fragment A reacted on immunoblots with purified receptors exactly at the position where the two monoclonal anti-ß antibodies reacted (FIG. 7D, lanes 1-3) and quantitatively precipitated intact ^{125}I-labeled IgE-receptor complex from unfractionated detergent extracts of RBL cells.

EXAMPLE 9

Biosynthetic Incorporation

Biosynthetic incorporation of labeled amino acids and monosaccharides was as described (Perez-Montfort et al. (1983) Biochemistry 27:5722-5728). The purification and analysis on gels and by immunoblotting of the IgE-receptor complexes have also been described (Rivera et al. (1988) Mol. Immunol.).

By using biosynthetic incorporation of two different amino acids labeled distinguishably, their ratio in the subunits of the receptor (Table 1, right part) was determined. The ratios of four distinctive amino acids to each other was in satisfactory agreement with the ratios predicted from the ß cDNA clone (Table 1, right part, columns 1-3). Because the cDNA for the ß subunit predicts three potential glycosylation sites, a double-labelling experiment using (^{3}H]mannose and (^{35}S)cysteine was also performed. Based on the relative carbohydrate data reported for the α subunit (Kaneilopoulos et al. (1980) J. Biol. Chem. 255:9060-9066) and correcting them on the basis of the peptide molecular weight for this

chain predicted from the cDNA, it was calculated that the α subunit contains ≈20 mol of mannose per mol. It was therefore possible to determine the mannose/cysteine ratio in the β subunit from the double-labeling experiment. The results showed only 0.05 mol/mol of cysteine or 0.3 mol/mol of the β subunit (Table 1, right part, column 4).

TABLE I. Amino Acid composition of ß Subunits

cDNA versus compositional analysis for the ß subunit																		
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Cys	Trp
Deduced from ß cDNA	20	12	23	24	15	12	19	17	4	15	36	9	12	1	8	8	6	2
Direct analysis*	22	13	22	27	13	19	18	14	4	13	31	7	10	2	10	10	5	ND
Double-labeling studies†																		

cDNA versus incorporation data				
	Met/His	Cys/His	Cys/Trp	Man/Cys
Deduced from ß cDNA	4	6	3	-
Direct analysis*				
Double-labeling studies†	4.2	5.1	2.5	0.05

*The mol % of each amino acid as reported by Alcaraz et al. ((1987) Biochemistry 26:2569-2575) was multiplied by 241 the number of residues, excluding tryptophan - predicted from the cDNA. ND, not determined.

†IgE-receptor complexes were purified from RBL cells incubated with a mixture of two precursors labeled with differentiable radioisotopes. The subunits were separated on a polyacrylamide gel. The gel was sectioned into 2-mm slices, extracted, and assayed for radioactivity by scintillation spectroscopy. The ratio of cpm of $^{35}S/^{3}H$ was individually calculated for α, ß, and γ subunits. The ratio in the α, subunit is proportional to the known molar ratio of the ^{35}S-labeled and ^{3}H-labeled residues in the α subunit. Hence, the corresponding ratio in the ß subunit (and the γ subunit) predicts the ratio of the same residues in the latter subunits.

SUBSTITUTE SHEET

EXAMPLE 10

Sequence Characteristics

There is ample evidence that the cDNAs that were isolated code for the ß subunit. (i) In vitro transcription of the cDNA and translation of the derived mRNA produce a protein whose apparent molecular weight on gel electrophoresis is indistinguishable from that of authentic ß chains (FIG. 7A). (ii) The cDNA accurately predicts the sequence of four peptides isolated from a tryptic digest of ß chains (FIG. 6A and SEQ ID NO:22) and a composition that agrees well with direct analyses and biosynthetic incorporations (Table I). (iii) Two monoclonal antibodies reactive with discrete epitopes on the ß subunit (Rivera et al. (1988) Mol. Immunol.) precipitate the protein synthesized in vitro from the cloned cDNA (FIG. 7A), and one of them reacts with a fragment of the protein expressed in *E. coli* (FIG. 7C). (iv) Polyclonal antibodies raised against a fragment of the ß subunit synthesized by *E. coli* transformants react with ß chains on immunoblots (FIG. 7D) and with IgE-receptor complex in solution.

The nucleotide sequence at the 5' end of the cloned cDNA (clone 1) does not in itself define the start of the open reading frame unambiguously. There is no leader sequence and no "in frame" stop codon preceding the presumptive start codon. In addition, the molecular weight deducted from the cDNA (M_r~27,000) is substantially lower than the one observed on $NaDodSO_4$ gels (M_r~32,000), although the ß subunit is not glycosylated. Therefore, it was possible that the start codon had been missed. Nevertheless, the aggregate data provide strong evidence that the full coding sequence for the ß subunit has been recovered. (i) Extensive attempts failed to reveal cDNAs in either of two separate libraries with a more extended 5' sequence. (ii) The major species generated by 5' extension studies terminated precisely at the point at which most of our clones started. (iii) The second ATG codon at the 5' end

meets the consensus characteristics of known initiation sites (Kozak (1987) Nucleic Acids Res. 15:8125-8148). That it is preceded by a nearby 5' ATG codon is uncommon, but not rare (Kozak (1987) Nucleic Acids Res. 15: 8125-8148), and has been observed for the human α subunit (Shimizu et al. (1988) Proc. Natl. Acad. Sci. USA 85:1907-1911; Kochan et al. (1988) Nucleic Acids Res. 16:3584). (iv) As already noted, *in vitro* translation of an mRNA transcribed from the cDNA containing only the second ATG codon gives a polypeptide indistinguishable in length from the authentic ß chains. An aberrant clone containing a start codon 48 nucleotides 5' to the presumed start codon directed the *in vitro* synthesis of a polypeptide with an apparent molecular weight appropriately greater than that of the β subunit. Therefore, the correspondence in apparent molecular weight between authentic ß chains and the protein synthesized *in vitro* from clone 1 is meaningful. The RNA transfer blotting data show an mRNa of ≈2.7Kb, precisely what would be anticipated from the cDNA that was sequenced (FIG. 6), given a poly (A) tail of ≈200 nucleotides. In the discussion that follows it is assumed that the ß chain begins with the methionine residue coded for by the second ATG and is, therefore, 243 residues long.

Only a single clone containing the *Cla I* restriction site was observed among the 37 clones analyzed. This clone likely resulted from a single base mutation during the cloning and is unlikely to represent a normally occurring mRNA. Conversely, six clones showing the deleted sequence (FIG. 6B) were observed and likely reflected an authentic species of mRNA. If translated, it would code for a M_r 14,000 protein with only a single transmembrane segment.

The sequence of the ß subunit contains potential sites for N-linked glycosylation at residues 5, 151, and 154. However, past and new incorporation data give no evidence for carbohydrate in the ß subunit (Perez-Montfort et al. (1983) Biochemistry 27:5722- 5728;

Holowka and Metzger (1982) Mol. Immunol. 19:219-227; and Table I). The sequence shows no unusual features or homology to previously reported sequences, in particular to those associated with Fc receptors or with Fc binding factors.

A hydropathicity analysis suggests that the ß subunit crosses the plasma membrane four times (FIG. 8). The hydrophilic NH_2 and COOH terminus would therefore be on the same side of the membrane. Expression of fragments of the ß cDNA indicate that mAbß-(NB) reacts within amino acids residues 149-243 (FIG. 7C) and that mAbß(JRK) reacts with fragment containing residues 1-21 (FIG. 7B). Because neither antibody reacts appreciably with intact cells but both react strongly with cell sonicates, the combined results are consistent with the NH_2 and COOH terminus being on the cytoplasmic side of the plasma membrane.

Earlier studies had suggested that the ß chain contained a M_r 20,000 "ß," domain resistant to proteolysis while membrane bound (Holowka and Metzger (1982) Mol. Immunol. 19:219-227). This portion also contained those residues that were modified by an intrabilayer labeling reagent (Holowka and Metzger (1982) Mol. Immunol. 19:219-227; Holowka et al. (1981) Nature (London) 289:306-808) and became linked to the ß and/or γ subunit when chemical crosslinking reagents were used (Holowka and Metzger (1982) Mol. Immunol. 19:219-227) and to the γ subunit when spontaneous disulfide linkage between the ß and γ_2, subunits occurred (Kinet et al. (1983) Biochemistry 22:5729-5732). The remainder, "$ß_2$,", appeared to contain the serine residues that became phosphorylated in situ (Perez-Montfort et al., (1983) Biochemistry 22:5733-5737; Quarto and Metzger (1986) Mol. Immunol. 23:1215-1223) but has never been positively identified as a discrete fragment. The sequence predicted by the cDNA for the ß subunit suggests that part or all of either the NH_2-terminal 59 residues or the COOH-terminal 44 residues, or of both, is cleaved off to generate the ß1 fragment.

EXAMPLE 11

Contransfection Experiments

The full-length coding sequences of the α and the ß subunits were cotransfected in COS 7 cells by using a vector for transient expression. No IgE-binding sites were expressed at the surface of transfected cells.

Studies of the receptor with low affinity for IgE on macrophages revealed a component that could be chemically crosslinked to the IgE-binding portion and that had an apparent molecular weight similar to the ß subunit of the high-affinity receptor (Finoloom and Metzger (1983) J. Immunol. 130:1489-1491). The peptides generated from this component by protease digestion appeared to differ from those released from ß subunits, but it raised the possibility that other Fc receptors also contained ß-like subunits that had heretofore escaped detection (Rivera et al. (1988) Mol. Immunol.). Evidence for this from RNA transfer blot experiments conducted at high stringency is not available. In particular, J774 cells are known to contain Fcγ receptors whose immunoglobul in-binding chain shows considerable homology to the α chain of the high-affinity receptor for IgE (Kinet et al. (1987) Biochemistry 26: 4605-4610). However, it was not possible to detect mRNA for ß chains by the methods that were employed. Similarly, NTD lymphoma cells gave negative results even though they have Fcγ receptors and show a low molecular weight component that reacts with mAbß(JRK) on immunoblots.

EXAMPLE 12

Isolation and Sequence Analysis of Peptides of the Gamma Subunit of FcεRI

FcεRI was purified by affinity chromatography using TNP-lysine beads as described in G. Alcaraz et al., Biochemistry 26:2569-2575 (1987). The eluate was applied to sepharose 4B beads coupled by cyanogen bromide to monoclonal anti-ß (JRK) (J. Rivera et al., Mol. Immunol. 25:647-661 (1988)). After washing the beads with 2 mM

CHAPS in borate buffered saline at pH8, the bound material was eluted at 65°C with 0.1% sodium dodecyl sulfate, phosphate buffered saline, pH 6.5. The subunits from FcεRI were then separated by HPLC size chromatography, the ß and γ containing fractions recovered, reduced, alkylated and digested with trypsin (J.-P. Kinet et al., Biochemistry 26:4605-4610 (1987)). The resulting peptides were separated by HPLC reverse phase chromatography as in J.-P. Kinet et al, Biochemistry 26:4605-4610 (1987). The chromatograms from the ß and γ digests were compared and the non-overlapping peptides were sequenced (J.-P. Kinet et al., Biochemistry 26: 4605-4610 (1987)).

EXAMPLE 13

Cloning and Sequencing of cDNA clones of the Gamma Subunit of FCεRI

Oligonucleotide probes were synthesized according to the sequences of peptide 3 (residues 41 to 47 of SEQ ID NO:27) and of peptide 4 (residues 54 to 62 of SEQ ID NO:27). The sequences were GA(A/G)AA(A/G)TCIGA(T/C)GCTCTCTA (SEQ ID NO:8) and AA(T/C)CA(A/G) GA(A/G)ACITA(T/C)GA(A/G)ACI(T/C)TIAA (SEQ ID NO:9). The methods used to screen the λgt11 library, to purify, subclone and sequence the positive clones are known in the art (J.P. Kinet et al., Biochemistry 26:4605-4610 (1987)). Peptide 3 and peptide 4 were also synthesized using a peptide synthesizer ABI 431A. The purity of the synthetic peptides was assessed by HPLC reverse phase chromatography, amino acid composition and mass spectroscopy. The peptides were conjugated either to ovalbumin using m-Maleimidobenzoyl-N-hydroxysuccinimide ester (F.T. Liu et al., Biochemistry 18:690-697 (1979)) at a molar ratio of 5:1 or to sepharose 4B with cyanogen bromide. Rabbits were immunized with the ovalbumin-conjugated peptides, the antisera collected and the antipeptide antibodies purified by affinity chromatography using sepharose 4B

conjugated peptides. The antipeptide antibodies were tested for reactivity with the γ subunit of FcεRI by Western blotting and for their ability to immunoprecipitate ^{125}I-IgE receptor complexes (J. Rivera et al., Mol. Immunol. 25:647-661 (1988)). The nucleotide sequence of the γ subunit of rat FcεRI (SEQ ID NO:26) obtained using the method of this invention, as well as the amino acid sequence (SEQ ID NO:27) that it predicts, are shown in FIG. 9.

In order to isolate and characterize the cDNA for the γ subunit, cDNAs for the FcεRI γ subunit were isolated from a λgt11 library prepared from rat basophilic leukemia (RBL) cells (J.P. Kinet et al., Biochemistry 26:4605-4610 (1987)) using oligonucleotide probes. Four peptide sequences were identified in a tryptic digest of the FcεRI γ subunits, and two of the peptides were used to synthesize two oligonucleotide probes (FIG. 9). The library was screened in duplicate with these two probes and overlapping plaques identified. Three discrete plaques were purified, subcloned and found to contain similar inserts of 0.6 to 0.7 kilobases (kb).

FIG. 9 shows the complete nucleotide sequence (SEQ ID NO:26) of the γ cDNA, the deduced amino acid sequence (SEQ ID NO:27) and the position in the sequence of the four original tryptic peptides. Analysis of the sequence (FIG. 10C) indicates an N-terminal hydrophobic signal peptide of 18 residues and a putative transmembrane domain separating a short extracellular portion of 5 residues from an intracytoplasmic domain. As predicted by earlier studies, the N-terminal processed γ subunit contains two cysteines, no methionine and no tryptophan residues (G. Alcaraz et al., Biochemistry 26:2569-2575 (1987)). Compositional analysis suggested that the γ subunit might contain one histidine residue (G. Alcaraz et al., Biochemistry 26:2569-2575 (1987)). However, biosynthetic dual labeling studies of the receptor using ^{35}S methionine and ^{3}H histidine, clearly indicated that no trace of histidine was incorporated into the receptor-associated γ subunit. Since the open reading frame

derived from three independent clones, each predicts a histidine six residues from the C-terminal end, it is expected that the γ subunit undergoes a C-terminal processing which clips off the histidine-containing segment. Furthermore, because the peptide immediately preceding this histidine was recovered (FIG. 9 and SEQ ID NO:26), the C-terminal segment must be cleaved after Lys 63. The predicted molecular weight of the fully processed γ would therefore be 7139 Da, in close agreement with values obtained for the purified reduced γ on sodium dodecyl sulfate - urea gels (G. Alcaraz et al, Biochemistry 26:2569-2575 (1987)).

Polyclonal antipeptide antibodies to a heptamer and to a nonamer peptide of the γ subunit (FIG. 9 and SEQ ID NO:27) were prepared and tested for reactivity with IgE receptor complexes for RBL cells. Both purified antipeptide antibodies reacted in a Western blot assay with the unreduced dimer and the reduced monomer of partially purified γ subunits. In addition, both antibodies quantitatively precipitated receptor-bound ^{125}I-IgE, either from an extract of RBL cells or from a preparation of partially purified receptors. Taken together, these results leave no doubt that the cDNAs isolated according to the present invention code for the γ subunit of FcεRI.

EXAMPLE 14
Expression of Receptor

In order to achieve expression of the receptor on the surface of COS 7 cells, the coding region of the α, β, and γ cDNAs were first subcloned separately into the SV 40 promoter-driven expression vector pSVL, prior to transfection into the COS-7 cells. The 810 bp EcoRI-Sty I restriction fragment of the α cDNA, the 965 bp EcoRI-EcoRV restriction fragment of the β cDNA and the 300 bp EcoRI-Dde I restriction fragment of the γ cDNA were subcloned separately into the Sma I site of the transient expression vector pSVL (Pharmacia, Uppsala, Sweden).

These restriction fragments individually contained the entire coding sequence of the appropriate subunit and variable portions of untranslated sequences. The only foreign sequence was the starting EcoRI recognition sequence which belonged to the initial linker. Cultured COS7 monkey kidney cells were then transfected with 40 μl of DNA by the standard calcium phosphate precipitation technique (L. Davis et al., in Basic Methods in Molecular Biology, ed. L. Davis, Elsevier, New York (1986)). After 48 hrs, the transfected cells (panels A and a of FIG. 11), as well as RBL cells (panels C and D of FIG. 11), were examined for surface expression of IgE binding by an IgE rosetting assay. The cells (5 x 10^6 cells/ml) were incubated at room temperature with (panels B and D) or without (panels A and C) μg/ml of non-specific rat IgE for 30 min and then with 5 μg/ml of anti-DNP-IgE (F.T. Liu et al., J. Immunol. 124:2728-2736 (1980)). The cells were then rosetted with ox red blood cells that had been modified with 2,4,6-trinitrobenzene sulfonic acid according to a known method (M. Rittenberg et al., Proc. Soc. Exp. Biol. Med. 132:575-581 (1969)). The results are shown in FIG. 11. FIG. 11A shows IgE-binding activity expressed by cells cotransfected with the α, β and γ subunits. Virtually all RBL cells, used as a positive control, formed rosettes (FIG. 11C). The rosettes were completely inhibited by preincubation of the cells with rat IgE (FIG. 11B and D) but not with human IgE (not shown). This coincides with the species specificity for the rat $Fc_\epsilon RI$ (A. Kulczycki et al., J. Exp. Med. 139:600-616 (1974))

In order to study the requirements for surface expression of IgE-binding activity, the cells were transfected with different combinations of the cDNAs for the three subunits, as shown in Table 2.

COS-7 cells were transfected with different combinations of cDNAs for the three subunits of $Fc_\epsilon RI$ (FIG. 11). The rosetting assay was performed for each transfection shown in Table 2. The assessment of the mRNA by Northern blotting was performed one time only (on

2×10^7 cells). Inhibitor was added to the cells in the experiments marked by an asterisk in Table 2 (50 μg/ml of non-specific rat IgE was added to the cells 30 minutes prior to the addition of the specific mouse anti-DNP IgE).

TABLE 2
Transfection Experiments

Cells	Transfections		Expression	
	cDNA	No.	Receptor mRNA	Ige Binding (rosettes/cells counted)
COS 7	0	9	0	0 / 12,948
	α	2	α	0 / 4,050
	αß	2	αß	0 / 3,504
	α	4	α	0 / 8,030
	ß	1	ß	0 / 2,069
	αß	29	αß	920 / 41,238
	αß	4	αß	0 / 7,542*
RBL	0	-	αß	"100%"

*Experiments where inhibitor was added.

Table 2 summarizes the data derived from all the transfection experiments performed as described up to here. The success rate of the transfection experiments has improved since that data was collected so that there is now routinely achieved 5±2% expression of IgE binding when α, ß and γ are simultaneously cotransfected.

Successful transfection was achieved for all combinations, as assessed by Northern blotting, but rosette forming cells were only detected after cotransfection of the full set of the cDNAs. These results indicate that the ß and γ subunits are required for surface-expression of the IgE-binding α subunit. It is further indicated that only the fully assembled receptor reaches the plasma membrane. This phenomenon has also been observed in other systems (M. McPhaul et al., Proc. Natl. Acad. Sci. USA 83:8863-8867 (1986); Y. Minami et al., Proc. Natl. Acad. Sci. USA 84:2688-2692

(1987)) and may be generally applicable to polymeric membrane proteins.

The easy dissociability of ß and γ, from α (B. Rivnay et al., Biochemistry 21:6922-6927 (1982)) has raised persistent uncertainty about whether conceptually, γ_2, and ß should be considered as subunits of $Fc_\epsilon RI$ or as "receptor associated" proteins. (An example of the latter is the CD3 complex which associates with the antigen receptor on thymus-derived lymphocytes (H. Clevers et al., Ann. Rev. Immunol. 6:629-662 (1988)). The subunit model for $Fc_\epsilon RI$ has been favored, for example, on the basis of the coordinate biosynthesis and catabolism of α, ß and γ, (R. Quarto et al., Molec. Immunol. 22: 1045-1052 (1985)). The new data on transfected cells obtained by the present invention provides the strongest evidence yet obtained that αßγ2 is the minimal structure for $Fc_\epsilon RI$.

The present model for the tetrameric $Fc_\epsilon RI$ receptor is illustrated in FIG. 12 and SEQ ID NOs. 28-30. In this model each of the 589 amino acid residues of which the expressed receptor is composed is shown as a circle. In the diagram, the exterior of the cell would be at the top, the plasma membrane in which the receptor is embedded would be in the middle, and the interior of the cell towards the bottom. Each of the polypeptide chains (the α, SEQ ID NO:28, on the left, the ß, SEQ ID NO:29, chain in the middle and the two γ, SEQ ID NO:30, chains on the right) contains one or more transmembrane segments.

The α chain (SEQ ID NO:28) is believed to contain two intrachain disulfide loops, and the sequences of these loops show considerable homology with immunoglobulins (J.P. Kinet et al., Biochemistry 26:4605 (1987); A. Shimizu et al, Proc. Natl. Acad. Sci. USA 85:1907 (1988); J. Kochan et al., Nucleic Acids Res. 16:3584 (1988)). Thus, the α subunit is another member of the immunoglobulin superfamily (A. Williams et al., Ann. Rev. Immunol. 6:381 (1988)). The extracellular and transmembrane segments of the α chain show considerable

homology with the immunoglobulin binding chain of Fc receptors that bind IgG (J. Ravetch et al., Science 234:178 (1986)), but the intracellular cytoplasmic tail is quite different. The carbohydrate residues that are covalently attached to the extracellular portion of the α chain are not indicated in FIG. 12. There are seven potential sites for N-linked carbohydrates (J.P. Kinet et al., Biochemistry 26:4605 (1987); A. Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907 (1988)), but which of these that are actually used by the cell remains to be determined. Studies show that the carbohydrate is not essential for the binding of IgE by this chain (B. Hempstead et al, J. Biol. Chem. 256:10717 (1981)).

The ß chain (SEQ ID NO:29) contains four transmembrane segments (J. P. Kinet et al., Proc. Natl. Acad. Sci. USA 85:6483 (1988)) and previous studies with monoclonal antibodies (J.P. Kinet et al., Proc. Natl. Acad. Sci. USA 85:6483 (1988); J. Rivera et al., Mol. Immunol. 25:647 (1988)) show that the amino- and carboxyltermini which are respectively 59 and 43 residues long, protrude from the cytoplasmic face of the plasma membrane. Similarly, the γ chains (SEQ ID NO:30) have an extensive intracellular extension but only very limited exposure to the exterior.

According to the general model, the putative transmembrane domains of the individual subunits are predicted from their respective hydropathicity plots (see FIG. 10, wherein a net free energy of > 20 kcal/mol for transfer to water suggests a transmembrane segment or a leader peptide (D. Engelman et al., Ann. Rev. Biophys. Biophys. Chem. 15:321-353 (1986)). These plots suggest one, four and one hydrophobic domains for the α, ß and each γ, respectively (i.e., seven transmembrane domains for the entire receptor). Members of a family of receptors interacting with G proteins also contain seven transmembrane domains (I. Herskowitz et al., Cell 50:995-996 (1987)). This family includes ß and α adrenergic, muscarinic receptors and rhodopsin. Although no sequence homology between FcεRI and these receptors is found, it

is significant that an interaction between FcRI and G proteins has been postulated to explain at least some of the biochemical pathways activated by this receptor (S. Cockcroft et al., Nature 314:534-536 (1985)). The topology of the α and β subunits has been discussed in J.P. Kinet et al., Biochemistry 26:4605-4610 (1987) and A. Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911 (1988), in particular, the cytoplasmic localization of the C- and N- terminal portions of the β subunit. Two pieces of evidence support the topology of the γ-dimer as shown in Fig. 12: The γ can be oxidatively iodinated on inverted vesicles but not on intact cells (D. Holowka et al.;, J. Biol. Chem. 259:3720-3728 (1984)) and, *in vivo*, γ becomes phosphorylated on threonine residues (R. Quarto et al., Mol. Immunol. 23:1215-1223 (1986)). None of the relevant residues are present in the small presumptive extracytoplasmic segment of γ but all are present on the presumptive cytoplasmic tail, i.e., two tyrosine and four threonine residues.

As a further means to examine the topology of the receptor, the putative extracellular and intracellular segments of the three subunits were analyzed for their relative content of basic residues, as suggested by G. von Heijne Biochim. Biophys. Acta 947:307-333 (1988). He found the ratio of basic/total residues varies as a function of the length of the segment studied, but in general was substantially higher in the non-translocated (intracellular) segments than in the translocated (extracellular) segments of membrane proteins. Table 3 below shows a good correspondence between the ratios calculated for the present model and the ratios expected on the basis of "known" membrane proteins (G. von Heijne, Biochim. Biophys. Acta 947:307-333 (1988)), thereby providing independent support for the topological model presented here.

TABLE 3

Ratio Lys + Arg/total in Translocated and Untranslocated Segments of Receptor Subunits

			Extracellular (translocated)			Intracellular (untranslocated)		
Polypeptide		No. residues	Ratio			No. residues	Ratio	
			found	expected			found	expected
α		179	0.13	0.11		22	0.31	0.19
ß	loop 1	17	0.06	0.04	N-term	59	0.10	0.10
	loop 3	28	0.03	0.04	loop 2	12	0.25	0.20
					C-term	43	0.12	0.18
γ		5	0	0.08		36	0.22	0.16
$\alpha\beta\gamma_2$		234	0.045	0.02-0.06		208	0.17	0.12-0.16

The expected values calculated from the data in FIG. 8 of G. von Heijne, Biochim. Biophys. Acta 947, 307-333 (1988), in which the ratio found for the extra-membrane segments from "known" proteins has been plotted as a function of the segments' length.

The model clarifies several important features with respect to the organization of the subunits. The ß and dimer of γ interact with each other; in detergent solutions they dissociate from 5the α as a unit before dissociating from each other (J. Rivera et al., Mol. Immunol. 25:647-661 (1988)), and occasionally, ß and the γ dimer are observed to be disulfide-linked to each other (J.P. Kinet, Biochemistry 22:5729-5732 (1983)). The likeliest candidates for this bond are γ-cys7 and ß-cys80 which are predicted to be topologically close. This would then require that at least the γ-cys26 residues are disulfide-linked in the γ dimer. Preliminary data on the receptor biosynthesis suggest that α and ß interact with each other.

The functional properties of $Fc_\epsilon RI$ are broadly similar to those of several $Fc_\gamma R$. $Fc_\gamma R$ appears to bind to homologous segments of the immunoglobulin's Fc region (B. Helm et al., Nature 331:180-183 (1988); A. Duncan et al., Nature 332:563-564 (1988)), and the binding site on the receptor is found on a homologous polypeptide having immunoglobulin-like domains (J.P. Kinet et al., Biochemistry 26:4605-4610 (1987); J. Ravetch et al.,

Science 234:718-725 (1986)). Both types of receptors need to be aggregated to initiate cell activation and, where studied, the latter appears to involve generation of broadly similar second messengers (H. Metzger et al., Ann. Rev. Immunol. 4:419-470 (1986); N. Hogg, Immunol. Today 9:185-187 (1988)). It is surprising, therefore, that whereas FcεRI consists of four polypeptide chains, seven transmembrane segments and five cytoplasmic segments, FcεRI appear to perform similar functions with a much simpler structure, i.e., an α-like subunit alone. The extreme case is that of FcεRIII which appears to lack even transmembrane and intracellular segments (P. Selvaray et al., Nature 333:565-567 (1988); D. Simmons et al., Nature 333:568-570 (1988); T. Huizinga et al., Nature 333:667-669 (1988)). It has been suggested that additional components of Fcγ receptors may have thus far been missed. Possibly such components are even more easily lost upon solubilization of the receptors than are the β and γ subunits of FRI (J.P. Kinet et al., Biochemistry 24:4117-4124 (1985)). A reasonable interpretation is that such hypothetical components would be homologous to β or γ or both. The availability of genetic probes for the latter components will now permit an in-depth exploration of this possibility.

The success in expression of IgE binding achieved according to the present invention has important therapeutic implications. Degranulation of mast cells and basophils triggered by FcεRI accounts for many of the symptoms of allergy. Given the high incidence of this disorder, the discovery of a specific inhibitor of IgE binding is expected to yield enormous therapeutic benefits. The development of such an inhibitor has been hampered by the lack of a practical *in vitro* assay for the binding of human IgE to the human receptors. For example, a recent assessment of IgE-derived peptides of their inhibitory capacity had to be determined by skintesting (B. Helm et al., Nature 331:180-183 (1988)), a cumbersome and potentially dangerous procedure.

That the present invention achieves the expression of the transfected rodent receptor indicates that human FcεRI can be similarly expressed. Alternatively, since at present only the cDNA coding for the human α subunit has been isolated (A. Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911 (1988); J. Kochan et al., Nucl. Acids Res. 16:3584 (1988)), it is expected that it can be expressed in cotransfections with the cDNAs coding for the rodent ß and γ chains.

A comparison between the human and rat α subunits is set forth in Table 4 below.

TABLE 4

Comparative Properties of Human and Rat Alpha Chains

Domain	Species		% Homology
	Human	Rat	
Extracellular	180	181	49
Transmembrane	21	21	67+
Intracellular	31	20	23
Total	232	222	47*
* Wt ave.			
+ Human: (amino acid residues 178-204 of SEQ ID NO:13)		WLQFFIPLLVVILFAVDTGLFISTQQQ	
Rat: (amino acid residues 179-205 of SEQ ID NO:12)		WLQLFPSLAVILFAVDTGLWFSTHKQ	

It may be seen from the above Table that there is an overall homology between the human and rat alpha chains of about 47%, but an almost 70% homology in the presumed transmembrane domains. Indeed, when the transmembrane domains are examined closely, there is a stretch of 10 consecutive residues that are completely identical. This stretch of consecutive residues is underlined in Table 4.

Because the transmembrane segment is the region of the α chain that is most likely to interact with the ß1 and γ chains, it was expected that the human α chain would be expressible, if transfected, along with the rat ß and γ chains. This has proved to be the case as the present inventors have been able to express human IgE binding by COS cells transfected simultaneously with the human α and the rat ß and γ subunits. It will be

advantageous, of course, to have permanently transfected cell lines and for such lines, one will want to utilize combinations of the IgE receptor subunits disclosed herein.

EXAMPLE 15

The Beta Subunit of FCεR Is Necessary for Expression in Mast Cells

FIGS. 20 and 21 present the results obtained from FACS analysis (IgE binding) of cells transfected with

FIG. 20: represents COS-7 transfected cells.

FIG. 21: represents KU812 cells (a basophil line).

The clone of Ku812 cells used does express the mRNA for the three subunits alpha, beta and gamma but the receptor is not naturally expressed on the surface.

In FIG. 20, the transfection of human alpha and gamma in COS-7 cells is confirmed to be sufficient for expression of the alpha-gamma complex on the surface of the transfectants. These results also show that human beta and not rat beta associates efficiently with human alpha and that therefore, rat beta cannot replace human beta.

FIG. 21 illustrates that transfection of alpha-gamma in KU812 results in very little expression of receptors. The level of expression is similar to the level obtained after transfection of beta and gamma. Therefore this level may be attributable to the endogenous alpha (for beta and gamma transfection) or to the endogenous beta (for alpha and gamma transfection). By contrast the level of expression after co-transfection of the three cDNAs is very substantial.

From these results, it may be concluded that:

1. in mast cells and basophils, regulation of the level of expression of the receptor may be different than in fibroblasts.

2. in human mast cells and basophils, receptor expression requires the presence of alpha, beta and

gamma; whereas in transfected fibroblasts, human alpha and gamma are sufficient.

EXAMPLE 16
Isolation, Mapping and Sequencing of the Human FceRI β Gene

Initial atempts to isolate human β cDNA clones were by screening a human mast cell cDNA library with full-length rat and mouse cDNA probes. These probes were radiolabeled and used to screen $7x10^5$ colonies. Four clones were isolated, all of which contained a 153 bp insert with 73% homology to rat β cDNA. The sequence of this insert corresponded to a portion of β which includes the intracellular loop and the third transmembrane domain. These four identical clones are the likely result of library amplification of a single clone generated by recombinations. Two additional libraries were screened: another mast cell cDNA library and a cDNA library derived from basophil-enriched leukocytes. The latter library was also used to isolate human γ cDNA clones. A total of 107 independent cDNA clones were screened with a panel of murine probes and oligonucleotides and with the 153 bp human β probe. However no additional clones were isolated.

$6x10^5$ independent genomic clones from a human genomic leukocyte library with the radiolabeled 153 bp human probe were subsequently screened, and 10 clones with an average size insert of 25 kb were isolated. These clones all hybridized with two 20 mer-oligonucleotide probes corresponding to the beginning and the end of the rat β coding γ sequence. Four different restriction patterns could be generated from the 10 clones. However, southern blots with various oligonucleotide probes scanning different regions of the rat β coding sequence indicated that the four restricted patterns were not the product of different genes. Rather the clones showed differences in the lengths of the sequences flanking the β gene.

One clone containing a 25 kb insert was chosen for further characterization mapping and sequencing. A

restriction map shown in Fig. 13 was constructed by complete and incomplete digestion with the restriction endonucleases Hind III, Pst I, BamH I, Xba I, Sma I and Kpn I. A 3.2 kb Hind III fragment was found to hybridize with oligonucleotide probes corresponding to the start codon, and transmembrane region I and II of rat β. A 2.8 kb Sma I fragment hybridized with rat β probes of transmembrane domain III and IV and a 4.5 kb Sma I fragment with probes of the stop codon region. The 3 fragments were subcloned into pGEM 3zf (+) or (-) and sequenced in full (FIG. 14 and SEQ ID NO:31). The fragment corresponding to the 0.9kb gap between the Hind III and 2.8 kb Sma I fragments was produced by PCR and sequenced. Analysis using PCR confirmed that the two Sma I fragments were adjacent to each other.

By comparing the sequences of the human β gene and the rat β cDNA (FIG. 15) seven homologous regions which were likely localized to correspond to seven different exons.

EXAMPLE 17

Synthesis of Human β cDNA Coding Sequence

In order to confirm the sequence of the exons and to define the intro-exon borders, human β cDNA was synthesized by reverse transcription of RNA purified from basophil-enriched leukocytes followed by an amplification of the reverse transcripts using the polymerase chain reaction (PCR) (described in Materials and Methods herein). This applied product extended from 2 nucleotides preceding the start codon to 32 nucleotides following the stop codon. The cDNA sequence was found to be identical to the corresponding sequence of the human β gene. This confirmed that the coding sequence of human β is contained in seven exons. Furthermore, the comparison of cDNA and gene sequences and the detection of consensus sequences for intron-exon borders in the human β gene allow for a precise determination of these

borders. The 5' borders of the six intervening introns invariably start with GT and the 3' borders end with AG.

EXAMPLE 18

Analysis of Human β Transcripts

To evaluate the length of 5' and 3' untranslated sequences, the size of human β transcripts was analyzed. RNA from basophil-enriched leukocytes obtained from different individuals were hybridized by northern blotting with the radiolabeled 153 bp human β probe (FIG. 16A) Two transcripts around 3.9 kb were found in human basophils but not in COS-7 cells. The human transcripts are substantially longer than their rodent counterparts (2.7 and 1.75 kb) (Ra. 1988, Kinet, 1988) as detected in RBL cells by crosshybridization. This longer size may explain initial failures to isolate human β cDNAs from the three oligo-dT primed libraries. Similar results were obtained with a full-length cDNA probe of human β. Hybridization of the same RNAs with a human α cDNA probe revealed transcripts for α of the expected size (1.1 kb) (FIG. 16B). RNA from different cell lines were also hybridized with a full length human β cDNA probe (FIG. 16C). The message for human β is only detected in the basophil line KU812 but not in U937, Daudi and Hela cells. An additional band is seen in KU812 which could correspond to unspliced transcripts.

With an open reading frame of 732 bp and assuming 200 bp for the poly A tail, human β transcripts should contain about 3 kb of untranslated sequences. FIG. 15 shows that most of the untranslated sequences are in the seventh exon. The possibility that additional exons of 3' or 5' untranslated sequences had not yet been identified was also explored.

EXAMPLE 19

A. Characterization of the (A) 5' End and of the Transcription Initiation Site

The transcription start site was determined by sequencing directly a PCR amplified product of the reverse transcribed RNA as described in "Experimental Procedures". RNA from basophil-enriched leukocytes was reverse transcribed from a primer of the human β coding sequence. Poly-A tails were added to the reverse transcripts by treatment with terminal transferase and the resulting cDNAs were amplified by PCR. Single stranded DNAs (positive strands poly dT tailed) were then produced by asymmetric PCR and directly sequenced. The cDNA sequence of the negative strand corresponding to the 5' end of the RNA is shown in FIG. 5A and is compared to the relevant sequence of the β gene. The perfect match between the two sequences ends after GGGTT. Then the cDNA sequence reproducibly shows a C, which is not present in the gene, followed by the expected poly-A tail. This additional C may correspond to the G of the cap structure and indicate the location of the start site.

Experiments of 5' extension (FIG. 17B) confirmed that there is a major start site in this area (about 11 nucleotides 3' of the position described above). It is difficult, though, to exclude the possibility that the faint bands seen below and above the major start sites correspond to minor start sits. However the presence of a TATAAA box found in the 5' sequence supports the existence of a unique start site. In addition the location of the TATAAA box (usually 25 nucleotides 5' of the start site) is more consistent with the precise localization of the start site as shown.

Indeed the TATAAA box is located between nucleotides 29 and 24 upstream of this start site as shown in FIG. 17A. Taken together the data indicate that the human β mRNA start with the sequence AACCC (see FIG. 14, SEQ ID NO:31, and FIG. 17A) and has 102 bp of 5' untranslated sequence.

B. Characterization of the 3' end

A comparison between the rat β cDNA and human β gene sequences FIG. 15) shows that the seventh exon of the β gene extends at least from nucleotides 6773 to at least nucleotide 8910. But an additional 3' untranslated sequence (about 800 bp) had to be found to fully account for the 3.9 kb transcripts. To analyze whether the missing sequence was part of the seventh exon or of other undetected exons, three probes from the β gene were prepared to test their reactivity with β transcripts. These transcripts hybridized in northern blots with both the NsiI-BamH1 fragment (nucleotides 8460-9250) and the BamHI-SphI fragment (nucleotides 9250-9714) but not with the fragment 3' of the SphI site. Interestingly two polyadenylation signals AATAAA are found at nucleotides 9663 and 9758 (FIG. 14 and SEQ ID NO:31). Therefore this region is likely to correspond to the end of exon 7. It is likely that both polyadenylation signals could be used to create the apparent doublet of transcripts around 3.9 kb (see FIG. 16).

EXAMPLE 20

Organization of the Human β Gene

Taken together the data presented herein indicate that the human β gene contains seven exons and six introns and spans about 10 kb. Exon 1 codes for 102 bp of 5' untranslated sequence and the first 18 amino acid residues of the N-terminal cytoplasmic tail. Exon 2 encodes the remaining of the cytoplasmic tail and the first three residue of TM1. Exon 3 codes for the remaining of TM1, the first extracellular loop and the first half of TM2. Exon 4 encodes the second half of TM2 and a portion of the cytoplasmic loop. Exon 5 codes for the last three residue of the cytoplasmic loop, TM3 and most of the second extracellular loop. Exon 6 codes for the last two residues of the extracellular loop, TM4 and the first quarter of the C-terminal cytoplasmic tail.

Finally, exon 7 codes for the remaining of the cytoplasmic tail and the long untranslated 3' sequence.

EXAMPLE 21

The Human β Protein

The human β protein comprises 244 amino-acid (aa) residues and has a molecular mass of 26,532 daltons (FIG. 18). Similar to rat (243 aa) and mouse β (236 aa), human β contains four hydrophobic segment suggestive of transmembrane domains (TM) but no leader peptide. FIG. 19 shows an alignment of the human sequence (SEQ ID NO:32) with the rat (SEQ ID NO:33) and mouse (SEQ ID NO:34) sequence. The consensus sequence for β (not shown) from the three species (rat, mouse and human) shows that 91.4% of the amino-acid residues are homologous while 68.7% are identical.

EXAMPLE 22

Transfection in COS-7 Cells:
Expression of Human and Hybrid FcεRI Receptors

Table 5

Functional expression of FcεRI after transfection of various subunit combinations

Transfected cDNAs			% Fluorescent cells (FACS)			
			n	Mean	±	SD
Human α	-	-		10.2		
Human α	Human β	-		10.2		
Human α	-	Human γ	7	10.4	±	8.7
Human α	Human β	Human γ		78.3	±	5.0
Human α	Rat β	Human γ		45.4	±	3.4
Rat α	Rat β	Rat γ	8	18.0	±	17.8
Rat α	Human β	Rat γ		102.4	±	2.0
Rat α	Human β	Human γ		51.8	±	1.3
Mouse α	Mouse β	Mouse γ		48.2	±	5.6
Mouse α	Human β	Mouse γ		61.6	±	1.2
Mouse α	Human β	Human γ		21.5	±	0.8
Human α	-	Rat γ_{trunc}		71.4	±	1.0
Human α	Rat β	Rat γ_{trunc}		53.2	±	2.8
Human α	Human β	Rat γ_{trunc}		77.4	±	7.9
Rat α	Rat β	Rat γ_{trunc}		29.3	±	0.8
Rat α	Human β	Rat γ_{trunc}		20.4	±	0.5

It was found that co-transfection of α, β, and γ cDNAs is necessary to promote expression of rat or mouse FcεRI on the surface of transfected COS-7 cells. By contrast, co-transfection of human α and ß cDNAs results in the surface expression of αγ complexes without apparent need for γ. With the availability of human γ cDNAs, the question was explored whether human β would influence in any way the efficiency of surface expression

of the human receptor complex. Table 5 shows that co-transfection of human α and γ cDNAs into COS-7 cells results in 10.4% ± 8.7 of the cells being fluorescent when analyzed by FACS after binding of fluoresceinated IgE. This level of expression is not significantly modified when human β cDNA is co-transfected with human α and γ cDNAs (8.3% ± 5.0). Thus, human β does not seem to influence the level of surface expression of human FceRI in transfected COS-7 cells. Substituting rat β or human β reduces the level of expression (5.4% ± 3.4).

The effect of substituting human β for rat β was analyzed. Co-transfection of rat α, β, γ cDNAs result in much higher level of expression (18.0% ± 17.8) than co-transfection of rat α, γ with human γ (2.5% ± 2.0) (Student's t statistic = 2.75; $p \leq 0.014$). Similarly co-transfection of mouse α, β, γ cDNAs is more efficient (8.2% ± 5.6) than co-transfection of mouse α, γ with human β (1.6% ± 1.2) (Student's t statistic:2.91; $p \leq 0.019$). Because replacing rat γ or mouse γ with human γ does not restore expression (compare 2.4% with 1.8%, and 1.6% with 1.5%), it is likely that the problem of expression resides in the human β-rat α or human β-mouse α interaction.

It is known that truncation of the cytoplasmic tail of rat γ prevents the surface expression of human α in transfectants (Varni-Blank, 1990). The question was whether human β could complement the surface expression of human α in these conditions. It was confirmed that co-transfection of human α with truncated rat γ permit only very poor surface expression of αγ complexes (1.4% ± 1.0). When human β is co-transfected with the latter combination there is an increase of expression (7.4% ± 7.3, n=7). However this increase does not become significant ($p \leq 0.035$) when one aberrant point is not included in the seven experiments. The same increase is not observed when rat β is substituted for human β (3.2% ± 2.8) suggesting again that there may be specific points of interaction between human α and β. In other experiments using the truncated rat γ, it was found that

human β cannot be substituted for rat β in its interaction with rat α (compare 9.3% ± 0.6 with 0.4% ± 0.4; ($t = 13.0$; $p \leq 0.006$).

Taken together these data indicate that there is a tendency for human β to interact more efficiently with human α than does rat β, but the species specificity is weak. By contrast, there is a strong species specificity in the interaction between rat β and rat α or between mouse β and mouse α.

Human αγ complexes may be expressed on the surface of transfected cells. Moreover co-transfection of human α and γ with rat β results only 20% of the receptors being αβγ complexes, the remaining 80% being αγ complexes. Therefore, it is theoretically possible that αγ complexes occur naturally. However in view of the species specificity of interaction between human β and α (see above), previous results obtained from the co-transfection of human α and γ with rat β suggest the *in vivo* situation could be different.

These genetic results, of course, provides much more than an assay, as important as the latter may be. Through directed mutation it will, in addition, allow the development of further information regarding the critical binding regions. It is expected that, using this information, rational drug design will become possible. It is further expected that it will be possible to block the function of the ·eceptor itself, i.e., it will be possible to interfere with the early biochemical signals that result from activation of the receptor.

EXAMPLE 23

Detection of a Candidate Inhibitor Substance

In still further embodiments, the present invention concerns a method for identifying new FcεRI inhibitory compounds, which may be termed as "candidate substances." It is contemplated that this screening technique will prove useful in general identification of any compounds that will serv e the purpose of inhibiting the formation

of $Fc_\epsilon RI$ as measured by various cell activation assays. (Mouse Interleukin-2 ELISA kit, Alberts et al., pp. 179-180, Adamczewski et al. (in press), Barones et al., 1991).

Thus, in these embodiments, the present invention is directed to a method for determining the ability of a candidate substance to inhibit the formation of the human $Fc_\epsilon RI$ complex, the method including generally the steps of:

(a) obtaining a composition comprising the human alpha, beta and gamma subunits of $Fc_\epsilon RI$ that are capable of complexing to form a functional and/or expressed receptor;

(b) admixing a candidate inhibitor substance with the composition; and

(c) determining the functional or expressed ability of the admixture.

An important aspect of the candidate substance screening assay hereof is the ability to prepare a composition of alpha, beta and gamma subunits in a relative purified form, for example, in a manner discussed herein. An aspect of the candidate substance screening assay in that without at least a relatively purified preparation, one will not be able to assay specifically for $Fc_\epsilon RI$ inhibition, as opposed to the effects of the inhibition upon other substances in the extract which then might affect the receptor. In any event, the successful cloning and isolation of the beta subunit now allows for the first time the ability to identify new compounds which can be used for inhibiting the $Fc_\epsilon RI$ in specific ways, thereby inhibiting the effects of the $Fc_\epsilon RI$ when bound to IgE.

The candidate screening assay is quite simple to set up and perform, and is related in many ways to the assays discussed above for determining $Fc_\epsilon RI$ activity. After obtaining a relatively purified preparation of the alpha, beta and gamma subunits, one will desire to simply admix a candidate substance with the preparation, preferably under conditions which would allow the receptor to form

but for inclusion of an inhibitory substance. Thus, for example, one will typically desire to use cell activation assays as indirect measures of the presence of a functional receptor, or receptor expression, or both.

Accordingly, one will desire to measure or otherwise determine the activity of the relatively purified receptor in the absence of the assayed candidate substance in order to assess the relative inhibitory capability of the candidate substance.

In still further embodiments, the present invention is concerned with a method of inhibiting receptor formation and/or function which include subjecting the subunits to an effective concentration of a candidate substance identified in accordance with the candidate screening assay embodiments. This is, of course, an important aspect of the invention in that it is believed that by inhibiting the receptor one will be enabled to treat or prevent various aspects of allergic reactions. It is believed that the use of such inhibitors to block the release of histamine by binding of IgE to $Fc_{\epsilon}RI$ and serve to treat or palliate the symptoms of an allergic response. Inhibitors may be useful by themselves or in conjunction with other therapies.

EXAMPLE 24

Identification and Use of $Fc_{\epsilon}RI$ Inhibitors

If the action of receptor of IgE is inhibited, the allergic reaction will not proceed. This inhibition may be either at the level of transcription, translation, or protein action. Interference with transcription would necessitate interference with mRNA formation on the DNA template. Preferably, interference with the translation would necessitate interfering with the synthesis of proteins on the mRNA template. Alternatively, the action of the receptor may itself be disrupted either by destroying the structure of the receptor, prohibiting its formation, or binding the receptor or components thereof irreversibly to inhibitors.

Specifically designed peptides which block the function of the receptor are extremely valuable in preventing and treating allergic diseases. Embodiments of these blockers (antagonists) include any substrate analogues or inhibitor, e.g., oligopeptides or their derivatives which contain the amino acid sequence of the IgE binding site. Methods for identifying suitable inhibitors form candidate substances are disclosed in Example 23.

EXAMPLE 25

Preparation of the Human ß Polypeptide by Recombinant Techniques

It is an additional object of the present invention to provide a ready means for producing the human beta subunit for use in detecting inhibitors, to develop treatment modalities, to develop antibodies for detection of the subunit, and to develop inactive mutants of the human beta subunits, which may also be use to inhibit formation of the $Fc_{\epsilon}RI$. Such mutants may be introduced into transgenic animals, for example, to produce animals useful for ß assays.

An exemplary embodiment for preparing the beta subunit protein is to prepare a nucleic acid segment which includes a nucleic acid sequence capable of encoding the desired protein or polypeptide. This segment may be that which encodes the entire subunit or only some portion of it, for example, the alpha or gamma binding domain of the subunit. The segment may be as small as that capable of triggering a positive signal with an antibody, thereby, identifying the presence of a beta subunit. Segments functionally equivalent to those shown in Fig. 14, may also be selected depending on the desired polypeptide to be produced. Functional equivalence may be determined by testing whether the segment can cause cell activation using techniques disclosed herein to detect inhibitors from among candidate substances.

The nucleic acid segment selected is transferred into an environment appropriate for expression of the segment as a polypeptide. This environment may be a vessel containing a mixture capable of inducing expression. Alternatively, the segment may be transferred to a host cell by transformation, transfection via a recombinant expression vector, electroporation, or a "gene gun." The host cell may be selected from CHO cells, T cells, KU812 cells, P815 cells, or the like.

The recombinant expression vector will generally include a promoter. Embodiments of promoters are the $\alpha 4$ promoter, or any other suitable prokaryotic or eukaryotic promotors.

EXAMPLE 26

Antibodies Against the Proteins of the Present Invention

In other embodiments, the invention concerns the preparation of antibodies to the beta subunit of $Fc_{\epsilon}RI$ and species derived therefrom, either recombinant or nonrecombinantly prepared.

Compositions which include monoclonal antibodies of the present invention may be prepared by first fusing spleen cells of a rodent with myeloma cells from the same rodent species, wherein the rodent providing the spleen cells have been immunized with the ß subunit peptide, precursor, or related peptides. The rodent species utilized will generally be a mouse. Of course, where a beta subunits is prepared which incorporates structural variations over the ones disclosed herein, it will likely be able to successfully employ a hybridoma system according to the species of interest.

In addition, the present invention provides a method for isolating beta subunits from other species which may be found antigenically cross-reactive with that of the human or rodent subunit. This method includes preparing an immunoabsorbent material having attached thereto an antibody to the subunit. Numerous immunoabsorbent

materials are known to those skilled in the art and include, for example, Affi-Gel, Cn-Sepharose, protein A=Sepharose, and numerous other well know immunoadsorbent techniques. All such techniques of the immuno cross-reactive species (for a more complete listing, see Monoclonal Hybridoma Antibodies: Techniques and Applications, John G. Hurrell, ed. CRC Press, 1982, incorporated herein by reference).

Materials and Methods

Screening of cDNA and genomic libraries

The human basophil cDNA library and the human leukocytes genomic library have been described before and are available (Kuster, 1990). The human lung cDNA library (Miller, 1989) and a human skin cDNA library were provided by L.B. Schwartz (Medical College of Virginia, Richmond).

The following probes were prepared for screening the various libraries: The EcoRI-EcoRV fragment of rat β (Kinet, 1988) and the EcoRI fragment of mouse β (Ra, 1989), both of which contain the entire coding sequence of β and part of the 3' untranslated region. Fragments of the coding region of rat β cDNA (bp 1-304) and mouse β cDNA (bp 433-708) were made by polymerase chain reaction (PCR). Multiple oligonucleotides corresponding to various regions of rat, mouse and human β were synthesized on a model 380A automated DNA synthesizer (Applied Biosystems, Foster City, CA). All double stranded DNA probes were radiolabeled by random primer labeling and the oligonucleotides by end labeling as described elsewhere (Davis, 1986).

Hybridization and washing conditions and procedures for plaque purification subcloning, sequencing and DNA analysis were as described previously (Kuster, 1990).

Southern blot analysis

Digestion of genomic DNA from five different individuals with BamH BgI II, Eco RI, Hind III, Msp I and Pvu II and hybridization of these digests with a human cDNA probe (from start to stop codon) supports the existence of a unique gene (FIG. 18). In addition the lengths of the restriction fragments detected on the southern blot are entirely consistent with the lengths predicted from the sequence of the gene. Three BamHI sites (nucleotide 156, 6908, 9250) are presenting the gene. As expected only one fragment (156-9250) is seen here because the other fragments should not hybridize with the cDNA probe. The two predicted Bgl II fragments (+334 to +1766 and +1766 to +7419) and the two predicted Hind III fragments (-454 to +2724 and +2724 to 100042) are readily detected. The results obtained after EcoRI and PvuII digestions are consistent the fact that none of these sites are found in the sequence of the gene. Finally the pattern observed after Msp I digestion is also consistent with predicted fragments of 2067 bp, 3870 bp and a larger 5' fragment extending from nucleotide 3622 to an undetermined Mspl site upstream of the gene.

cDNA synthesis by using the Polymerase Chain Reaction (PCR)

Basophils from 240ml of blood were purified by double Percoll gradients as previously described (Warner, 1987) and basophil RNA extracted by the guanidium isothiocyanate method (Davis, 1986). Two μg of total RNA were reverse transcribed with Superscript reverse transcriptase using a random 9-mer primer as recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg MD). One twentieth of the reaction product was amplified using the following primers: a 23-mer complementary to nucleotide -2 to +21 of the human β coding sequence and as backward primer a degenerated 21-mer of the mouse and rat β sequences starting 32 nucleotides after the stop codon. Temperature cycles were as follows: 1 cycle of 2 min. 95° / 2 min. 94° / 5

min. 37° / 40 min. 72°, 4 cycles of 40 sec. 94° / 1 min. 37° / 4 min. 72°, and 36 cycles of 40 sec. 94° / 1 min. 50° / 4 min. 72° followed by a single 15 minute extension. One µl of this reaction was reamplified omitting cycles 2 to 5 and the amplification product subcloned into pCR1000 using the TA cloning kit (Invitrogen, San Diego, CA).

Direct sequencing of gene fragments obtained by PCR

Purified insert-containing phage DNA from the leukocyte genomic library was linearized with NotI and 100ng amplified with primers flanking the region to be sequenced. DNA amplification was achieved using 40 of the following cycles: denaturation for 1 min. at 94°C, annealing or 2 min. at 45-50°C and extension for 3-6 min. at 72°C. Subsequently 1 µl of the amplified material was reamplified in three separate reactions (50 µl) Under identical conditions omitting one of the 2 primers in order to generate single stranded DNA. The three reactions were pooled, applied to an Ultrafree MC 30.000 spin column (Millipore, Bedford MA), and washed four times before being evaporated by vacuum. The single stranded DNA was sequenced by using the omitted primer or an internal primer. The comparison of sequences obtained by this method or by sequencing non amplified fragments being subcloned in pGEM vectors revealed no differences.

Sequencing the transcription start site

PCR was used to define the transcription start site. Procedures published elsewhere (Frohman, 1987) were modified as follows: 5 µg RNA were reverse transcribed as detailed above by using a primer corresponding to nucleotide +451 to 429 of the coding region. The resulting product was washed on a Centricon 100 column (Amicon, Beverly MA) and a poly-A tails were at both ends addedusing terminal transferase (Bethesda Research Laboratories, Gaithersburg MD) as recommended by the manufacturer. One sixth of this reaction was amplified with the following 2 primers: a 33-mer consisting of the

M13 primer sequence followed by 17 T's and for the 3' end a primer derived from nucleotide 331 to 308 of the human β coding region sequence. Subsequently an internal amplification was performed exchanging the 3' primer for one equivalent to nucleotide +189 to 169. Finally, single stranded DNA was produced for sequencing by using an oligonucleotide corresponding to nucleotide 54 to 33 as the only primer. For all PCR's the annealing temperature was 45°C, the extension time 3 min.

Analysis of the transcription start site by 5' extension

An end labeled oligonucleotide corresponding to the negative strand at nucleotide 54 to 33 after the start codon was hybridized overnight at 42°C to either 10 µg total RNA from enriched basophils or 10 µg tRNA, followed by extension with Superscript reverse transcriptase (Bethesda Research Laboratories, Gaithersburg MD) at 45°C for 90 min. The primer-extended products were separated on a 5% polyacrylamide urea gel in parallel with the sequencing reactions of the genomic DNA.

Cell Line KU812

A new myeloid cell line (KU812) was established from a patient with blastic crisis of chronic myelogenous leukemia. His blasts were morphologically characteristic of immature basophils and basophil colonies were grown in agar culture of the blood mononuclear cells. Suspension culture of his blood cells was continue for more than 2.5 years. The KU812 cells morphologically showed a fine reticular nuclei with nucleoli, and some of them contained metachromatic granules with toluidine blue (TB) staining. These granules were positive for astra blue (AB) staining. Immunological marker studies revealed that there were no lymphoid characters except Fc receptors. The KU812 cells grew colonies in in vitro agar cultures, which were proved to be composed of basophils by TB staining and AB staining. Cytogenetic analysis showed marked aneuploidy and was positive for the Philadelphia chromosome (Ph^1). The cell lysate was

proved to contain histamine. These data suggest that KU812 is a cell line from leukemic basophil precursors. This is the first human basophil cell line. KU812 is useful in clarifying the mechanism of basophilic differentiation of the stem cells.
(Kishi, Leuk, Res, 1985, a: 381-390).

Other methods

Northern and Genomic Southern blots were performed as described elsewhere (Davis, 1986). The various cDNAs were subcloned into the eukaryotic expression vector pCDL-SR(α) for the transfection studies (Takebe, 1988). COS-7 cells were transfected by the standard DEAE-Dextran method (Maniatis, 1982), except that a 3 minute incubation of the transfected cells in 10% DMSO in media as added after the chloroquine treatment.

While the invention has been described with respect to certain specific embodiments, it will be appreciated that many modifications and changes may be made by those skilled in the art without departing from the spirit of the invention. It is intended, therefore, by the appended claims to cover all such modifications and changes as fall within the true spirit and scope of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: KINET, Jean-Pierre

(ii) TITLE OF INVENTION: ISOLATION, CHARACTERIZATION, AND USE OF THE HUMAN B SUBUNIT OF THE HIGH AFFINITY RECEPTOR FOR IMMUNOGLOBULIN

(iii) NUMBER OF SEQUENCES: 34

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Alexandria
(D) STATE: VA
(E) COUNTRY: USA
(F) ZIP: 22313-0299

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/869,933
(B) FILING DATE: 16-APR-1992
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: 40399/154 NIHD

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(C) TELEX: 899149

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGTACTGGC TATGATTTTT TATCCCATTG 30

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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AAGTACTGGC TATGATTTTT TATCCCATTG 30

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(B) TYPE: amino acid
(D) TOPOLOGY: linear

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1 5 10 15

Thr

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(ix) FEATURE:
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(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

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(B) LOCATION: 15
(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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(2) INFORMATION FOR SEQ ID NO:6:

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(B) TYPE: nucleic acid
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(ix) FEATURE:
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(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

(ix) FEATURE:
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(B) LOCATION: 15
(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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(2) INFORMATION FOR SEQ ID NO:7:

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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(2) INFORMATION FOR SEQ ID NO:8:

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(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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(2) INFORMATION FOR SEQ ID NO:9:

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(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 12
(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 21
(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 24
(D) OTHER INFORMATION: /note= "N in this sequence represents inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAYCARGARA CNTAYGARAC NYTNAA 26

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1174 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 107..880

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
TACTAAGAGT CTCCAGCATC CTCCACCTGT CTACCACCGA GCATGGGCCT ATATTTGAAG      60

CCTTAGATCT CTCCAGCACA GTAAGCACCA GGAGTCCATG AAGAAG ATG GCT CCT         115
                                                   Met Ala Pro
                                                     1

GCC ATG GAA TCC CCT ACT CTA CTG TGT GTA GCC TTA CTG TTC TTC GCT         163
Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu Phe Phe Ala
  5                  10                  15

CCA GAT GGC GTG TTA GCA GTC CCT CAG AAA CCT AAG GTC TCC TTG AAC         211
Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val Ser Leu Asn
 20                  25                  30                  35

CCT CCA TGG AAT AGA ATA TTT AAA GGA GAG AAT GTG ACT CTT ACA TGT         259
Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr Leu Thr Cys
                 40                  45                  50

AAT GGG AAC AAT TTC TTT GAA GTC AGT TCC ACC AAA TGG TTC CAC AAT         307
Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp Phe His Asn
             55                  60                  65

GGC AGC CTT TCA GAA GAC ACA AAT TCA AGT TTG AAT ATT GTG AAT GCC         355
Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile Val Asn Ala
         70                  75                  80
```

```
AAA TTT GAA GAC AGT GGA GAA TAC AAA TGT CAG CAC CAA CAA GTT AAT        403
Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln Gln Val Asn
 85                  90                  95

GAG AGT GAA CCT GTG TAC CTG GAA GTC TTC AGT GAC TGG CTG CTC CTT        451
Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp Leu Leu Leu
100                 105                 110                 115

CAG GCC TCT GCT GAG GTG GTG ATG GAG GGC CAG CCC CTC TTC CTC AGG        499
Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu Phe Leu Arg
            120                 125                 130

TGC CAT GGT TGG AGG AAC TGG GAT GTG TAC AAG GTG ATC TAT TAT AAG        547
Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys
        135                 140                 145

GAT GGT GAA GCT CTC AAG TAC TGG TAT GAG AAC CAC AAC ATC TCC ATT        595
Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile
    150                 155                 160

ACA AAT GCC ACA GTT GAA GAC AGT GGA ACC TAC TAC TGT ACG GGC AAA        643
Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys
165                 170                 175

GTG TGG CAG CTG GAC TAT GAG TCT GAG CCC CTC AAC ATT ACT GTA ATA        691
Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile Thr Val Ile
180                 185                 190                 195

AAA GCT CCG CGT GAG AAG TAC TGG CTA CAA TTT TTT ATC CCA TTG TTG        739
Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu
            200                 205                 210

GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA TTA TTT ATC TCA ACT CAG        787
Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile Ser Thr Gln
        215                 220                 225

CAG CAG GTC ACA TTT CTC TTG AAG ATT AAG AGA ACC AGG AAA GGC TTC        835
Gln Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe
    230                 235                 240

AGA CTT CTG AAC CCA CAT CCT AAG CCA AAC CCC AAA AAC AAC TGATATAATT     887
Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn Asn
245                 250                 255

ACTCAAGAAA TATTTGCAAC ATTAGTTTTT TTCCAGCATC AGCAATTGCT ACTCAATTGT     947

CAAACACAGC TTGCAATATA CATAGAAACG TCTGTGCTCA AGGATTTATA GAAATGCTTC    1007

ATTAAACTGA GTGAAACTGG TTAAGTGGCA TGTAATAGTA AGTGCTCAAT TAACATTGGT    1067

TGAATAAATG AGAGAATGAA TAGATTCATT TATTAGCATT GTAAAAGAGA TGTTCAATTT    1127

CAATAAAATA AATATAAAAC CATGTAAAAA AAAAAAAAAA AAAAAAA                  1174
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 257 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu
 1               5                  10                  15

Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val
            20                  25                  30

Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr
        35                  40                  45

Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp
    50                  55                  60

Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile
65                  70                  75                  80

Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln
                85                  90                  95

Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp
            100                 105                 110

Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu
        115                 120                 125

Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile
    130                 135                 140

Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn
145                 150                 155                 160

Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys
                165                 170                 175

Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile
            180                 185                 190

Thr Val Ile Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile
        195                 200                 205

Pro Leu Leu Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile
    210                 215                 220

Ser Thr Gln Gln Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg
225                 230                 235                 240

Lys Gly Phe Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn
                245                 250                 255

Asn
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 222 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat
(B) STRAIN: FcRI alpha subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Ala Thr Gln Lys Ser Val Val Ser Leu Asp Pro Pro Trp Ile Arg Ile
1               5                  10                  15

Leu Thr Gly Asp Lys Val Thr Leu Ile Cys Asn Gly Asn Asn Ser Ser
            20                  25                  30

Gln Met Asn Ser Thr Lys Trp Ile His Asn Asp Ser Ile Ser Asn Val
        35                  40                  45

Lys Ser Ser His Trp Val Ile Val Ser Ala Thr Ile Gln Asp Ser Gly
    50                  55                  60

Lys Tyr Ile Cys Gln Lys Gln Gly Phe Tyr Lys Ser Lys Pro Val Tyr
65                  70                  75                  80

Leu Asn Val Met Gln Glu Trp Leu Leu Leu Gln Ser Ser Ala Asp Val
                85                  90                  95

Val Leu Asp Asn Gly Ser Phe Asp Ile Arg Cys Arg Ser Trp Lys Lys
            100                 105                 110

Trp Lys Val His Lys Val Ile Tyr Tyr Lys Asp Asp Ile Ala Phe Lys
        115                 120                 125

Tyr Ser Tyr Asp Ser Asn Asn Ile Ser Ile Arg Lys Ala Thr Phe Asn
    130                 135                 140

Asp Ser Gly Ser Tyr His Cys Thr Gly Tyr Leu Asn Lys Val Glu Cys
145                 150                 155                 160

Lys Ser Asp Lys Phe Ser Ile Ala Val Val Lys Asp Tyr Thr Ile Glu
                165                 170                 175

Tyr Arg Trp Leu Gln Leu Ile Phe Pro Ser Leu Ala Val Ile Leu Phe
            180                 185                 190

Ala Val Asp Thr Gly Leu Trp Phe Ser Thr His Lys Gln Phe Glu Ser
        195                 200                 205

Ile Leu Lys Ile Gln Lys Thr Gly Lys Gly Lys Lys Lys Gly
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: homo sapien
(B) STRAIN: FcRI alpha subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile
1               5                  10                  15

Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe
            20                  25                  30
```

Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu
35 40 45

Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly
50 55 60

Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro Val Tyr
65 70 75 80

Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala Glu Val
85 90 95

Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn
100 105 110

Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys
115 120 125

Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr Val Glu
130 135 140

Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu Asp Tyr
145 150 155 160

Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala Pro Arg Glu Lys
165 170 175

Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala
180 185 190

Val Asp Thr Gly Leu Phe Ile Ser Thr Gln Gln Gln Val Thr Phe Leu
195 200 205

Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe Arg Leu Leu Asn Pro His
210 215 220

Pro Lys Pro Asn Pro Lys Asn Asn
225 230

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 227 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: FcRI alpha subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ala Thr Glu Lys Ser Val Leu Thr Leu Asp Pro Pro Trp Ile Arg Ile
1 5 10 15

Phe Thr Gly Glu Lys Val Thr Leu Ser Cys Tyr Gly Asn Asn His Leu
20 25 30

Gln Met Asn Ser Thr Thr Lys Trp Ile His Asn Gly Thr Val Ser Glu
35 40 45

Val Asn Ser Ser His Leu Val Ile Val Ser Ala Thr Val Gln Asp Ser
50 55 60

```
Gly Lys Tyr Ile Cys Gln Lys Gln Gly Leu Phe Lys Ser Lys Pro Val
65                  70                  75                  80

Tyr Leu Asn Val Thr Gln Asp Trp Leu Leu Leu Gln Thr Ser Ala Asp
                85                  90                  95

Met Ile Leu Val His Gly Ser Phe Asp Ile Arg Cys His Gly Trp Lys
            100                 105                 110

Asn Trp Asn Val Arg Lys Val Ile Tyr Tyr Arg Asn Asp His Ala Phe
        115                 120                 125

Asn Tyr Ser Tyr Glu Ser Pro Val Ser Ile Arg Glu Ala Thr Leu Asn
    130                 135                 140

Asp Ser Gly Thr Tyr His Cys Lys Gly Tyr Leu Arg Gln Val Glu Tyr
145                 150                 155                 160

Glu Ser Asp Lys Phe Arg Ile Ala Val Val Lys Ala Tyr Lys Cys Lys
                165                 170                 175

Tyr Tyr Trp Leu Gln Leu Ile Phe Pro Leu Leu Val Ala Ile Leu Phe
            180                 185                 190

Ala Val Asp Thr Gly Leu Leu Leu Ser Thr Glu Glu Gln Phe Lys Ser
        195                 200                 205

Val Leu Glu Ile Gln Lys Thr Gly Lys Tyr Lys Lys Val Glu Thr Glu
    210                 215                 220

Leu Leu Thr
225
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 9..11

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 21..32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
GAATTAAT ATG AATGAATTT AAG GTC TCC TTG                              32
         Met           Lys Val Ser Leu
           1             1
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met
1

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Lys Val Ser Leu
1

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 9..38

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTAAT ATG GTC CCT CAG AAA CCT AAG GTC TCC TTG 38
Met Val Pro Gln Lys Pro Lys Val Ser Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Val Pro Gln Lys Pro Lys Val Ser Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
AAG TAC TGG CTA TGATTTTTTA TCCCATTG                                    30
Lys Tyr Trp Leu
  1               5
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Lys Tyr Trp Leu
  1
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2545 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..786

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 46..54

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 55..786

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
ACGTTTCTGT GTAACAATAT CTTTTATTCC TGGATAGTCC AATTA ATG AAA AAA          54
                                                  Met Lys Lys
                                                   -3

ATG GAC ACA GAA AAT AAG AGC AGA GCA GAT CTT GCT CTC CCA AAC CCA        102
Met Asp Thr Glu Asn Lys Ser Arg Ala Asp Leu Ala Leu Pro Asn Pro
  1               5                  10                  15

CAA GAA TCC CCC AGC GCA CCT GAC ATT GAA CTC TTG GAA GCG TCC CCT        150
Gln Glu Ser Pro Ser Ala Pro Asp Ile Glu Leu Leu Glu Ala Ser Pro
            20                  25                  30

CCT GCA AAA GCT CTA CCA GAG AAG CCA GCC TCA CCC CCA CCA CAG CAG        198
Pro Ala Lys Ala Leu Pro Glu Lys Pro Ala Ser Pro Pro Pro Gln Gln
        35                  40                  45
```

```
ACA TGG CAG TCA TTT TTG AAG AAA GAG TTG GAG TTC CTG GGC GTA ACC        246
Thr Trp Gln Ser Phe Leu Lys Lys Glu Leu Glu Phe Leu Gly Val Thr
     50                  55                  60

CAA GTT CTG GTT GGT TTG ATA TGC CTT TGT TTT GGA ACA GTT GTC TGC        294
Gln Val Leu Val Gly Leu Ile Cys Leu Cys Phe Gly Thr Val Val Cys
 65                  70                  75                  80

TCC ACA CTC CAG ACT TCA GAC TTT GAC GAC GAA GTG CTT TTA TTA TAT        342
Ser Thr Leu Gln Thr Ser Asp Phe Asp Asp Glu Val Leu Leu Leu Tyr
                 85                  90                  95

AGA GCA GGC TAC CCA TTC TGG GGT GCA GTG CTG TTT GTT TTG TCT GGA        390
Arg Ala Gly Tyr Pro Phe Trp Gly Ala Val Leu Phe Val Leu Ser Gly
            100                 105                 110

TTT TTG TCA ATT ATG TCC GAA AGG AAA AAC ACA CTG TAT CTG GTG AGA        438
Phe Leu Ser Ile Met Ser Glu Arg Lys Asn Thr Leu Tyr Leu Val Arg
        115                 120                 125

GGC AGC CTG GGA GCA AAC ATT GTC AGC AGC ATC GCT GCA GGC TTG GGG        486
Gly Ser Leu Gly Ala Asn Ile Val Ser Ser Ile Ala Ala Gly Leu Gly
    130                 135                 140

ATC GCC ATA TTG ATT CTC AAT CTG AGC AAC AAC TCC GCT TAT ATG AAC        534
Ile Ala Ile Leu Ile Leu Asn Leu Ser Asn Asn Ser Ala Tyr Met Asn
145                 150                 155                 160

TAC TGC AAG GAT ATA ACC GAA GAC GAT GGT TGC TTC GTG ACT TCT TTC        582
Tyr Cys Lys Asp Ile Thr Glu Asp Asp Gly Cys Phe Val Thr Ser Phe
                165                 170                 175

ATC ACA GAA CTG GTG TTG ATG TTG CTG TTT CTC ACC ATC CTG GCC TTT        630
Ile Thr Glu Leu Val Leu Met Leu Leu Phe Leu Thr Ile Leu Ala Phe
            180                 185                 190

TGC AGT GCC GTG CTG CTC ATT ATC TAT AGG ATT GGA CAA GAA TTT GAG        678
Cys Ser Ala Val Leu Leu Ile Ile Tyr Arg Ile Gly Gln Glu Phe Glu
        195                 200                 205

CGT AGT AAG GTC CCC GAT GAC CGT CTC TAT GAA GAA TTA CAT GTG TAT        726
Arg Ser Lys Val Pro Asp Asp Arg Leu Tyr Glu Glu Leu His Val Tyr
    210                 215                 220

TCA CCA ATT TAC AGT GCG TTG GAA GAC ACA AGG GAA GCG TCC GCA CCA        774
Ser Pro Ile Tyr Ser Ala Leu Glu Asp Thr Arg Glu Ala Ser Ala Pro
225                 230                 235                 240

GTG GTT TCA TAAGAATCAA GGGGCCAGGA CAATCTGATT CCAGTCTAGT                823
Val Val Ser
```

CTTGAGAGTC GATCTTTTTG CAACATTATG GCAACATTTC TGTTTCCTCC GCACTCTATC 883

AACTTTTCAA TTGGATTGTT CTGTAGATAC CCCTGTTTCA GTTATGATGC CTCTGGTCTT 943

TAATTATCTC CCTTTTTGTG GATATCGTTC AATCCAGTTT TCTTGTTTTG TGTCACAGTC 1003

TCACATACAA CCTTTCTGGA AAGTCATCAA AAACAAGCTA GCTTTTATTG CATGTCTACT 1063

TTCATGAACA AAAGGAAGGA GGAGTTATTT TGAGAGTTTA ACTAAACTTA GATAATCAGG 1123

TAATATTTGA CTCTTAGTTC ATTTTAGAAT TCTCAACAAT ACTTGTGCAT GATATATGCC 1183

CACCATATCA AGCCTTCTAT ATATATTTAA TATGGTATTT ACTTTTCTAT GTAGATAGAT 1243

TTTCCACCCT CAATAATAAT GGGTTTTTCA GAGACATAAA GCTTTATGAA AAGACACATA 1303

TTATCTAATT CATGGGTATA TTCACTAATA CAGTTGTTGC TCAGTGGTGT TTACTACTTG 1363

GTGGGTAGTA GGTAATAGAG AACATTATTA AATCATTCAG TGTAGTGAGA TGCATAGGTA 1423

AAATCAGGGA CACTGTGAGT GTGTATATCT TTTGGTAAGA CATGTGTGAA AATGAAGAAT 1483

AAACTGATGA AGACTTGAGC TGGAAAGTAG TCAATGGGAA TGACAAGAAA TGATTGTGTA 1543

TAACACTTGT AGATAAATAA CTACCAACAA TTGGTAGAGA TTGCCATGTA TGCCTAAAAT 1603

CTCCCAGCCC AAGGCCAGCC TCTGTTACAC AGTGAGTTAG AGGCCAGTCT GGGCTACACA 1663

AGATCATACA TCAAAGGACG AAAGAAGATG TTGGTTCAAA CTGTTAACAC AGTAAGGGAT 1723

ATTTAAACAA ACAGAAGTTT GACTGATATA TTGAGTGCTT GAGTTTTTAA TAAAACTGAA 1783

TGAATAACAT TGCGGGGGAG GGGAGCAGTG ATGCAGAAGT CTGGATGATG GAGGAGTAGC 1843

AGAATCAGAT GAAACATTGA AACGTATTTC CAGACTTTTG TTCTGAGATG GTTATAAGAG 1903

CAATCACCAT TAAATGAAGA AGGTCAAGAC ACCAAAAGAA TTATTTTGAG ATAGAATTAA 1963

GACAGTCAAA ATCCACATGC CTATACTTAG AAGGTGAAGT AAGGATCAAA AGTAGAAAGC 2023

CTAACGATTA GTTGGAAAAG CATATTACGT TAGGCAGCAG ATGTCTATAG TGGAGAAAAG 2083

TTAAACAAGG AGAAATAATG AACCACCAGA GACTCTACAT GTTGGTTTGG GAAATAAGAG 2143

AAAATAGCAA TTCTAAACGA ATGCAAACTC TGAAGAAGCA TTTCCCAAAG GGTGTGGGCA 2203

GAGGACCAGA ACATTTGCAA ATGTACCTAG AGAGCAAACC TGAATAGGAG GTAAAATGGG 2263

GGAAAAGCAG CTAAGAAAAT GATTTTGTTG CTGTTATTTA GATTTTAAAA GAAACAAAAA 2323

GAGTCATTAA AAATCTGTTT GCTGGGATCA GTTATTGTGT TCTCTGTGTA TGTCCAAAGT 2383

ACAGGTAACT TTTCTAAATC TTCCTGTAAG GCTCACCTCA TATGTCTCTT CACATAGCCA 2443

CACCCTTGAT TCACAGTTAC TCTACCACAG TAGTAAACTG TGCTTGTGGT CTCCCTTATG 2503

TATCTTCACT AGTGTTTATA AAATAAATCA GAATTATTTA AA 2545

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 246 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Lys Lys Met Asp Thr Glu Asn Lys Ser Arg Ala Asp Leu Ala Leu
 -3          1               5                  10

Pro Asn Pro Gln Glu Ser Pro Ser Ala Pro Asp Ile Glu Leu Leu Glu
     15                  20                  25

Ala Ser Pro Pro Ala Lys Ala Leu Pro Glu Lys Pro Ala Ser Pro Pro
 30                  35                  40                  45

Pro Gln Gln Thr Trp Gln Ser Phe Leu Lys Lys Glu Leu Glu Phe Leu
                 50                  55                  60
```

```
Gly Val Thr Gln Val Leu Val Gly Leu Ile Cys Leu Cys Phe Gly Thr
            65                  70                  75

Val Val Cys Ser Thr Leu Gln Thr Ser Asp Phe Asp Asp Glu Val Leu
        80                  85                  90

Leu Leu Tyr Arg Ala Gly Tyr Pro Phe Trp Gly Ala Val Leu Phe Val
     95                 100                 105

Leu Ser Gly Phe Leu Ser Ile Met Ser Glu Arg Lys Asn Thr Leu Tyr
110                 115                 120                 125

Leu Val Arg Gly Ser Leu Gly Ala Asn Ile Val Ser Ser Ile Ala Ala
                130                 135                 140

Gly Leu Gly Ile Ala Ile Leu Ile Leu Asn Leu Ser Asn Asn Ser Ala
            145                 150                 155

Tyr Met Asn Tyr Cys Lys Asp Ile Thr Glu Asp Asp Gly Cys Phe Val
        160                 165                 170

Thr Ser Phe Ile Thr Glu Leu Val Leu Met Leu Leu Phe Leu Thr Ile
    175                 180                 185

Leu Ala Phe Cys Ser Ala Val Leu Leu Ile Ile Tyr Arg Ile Gly Gln
190                 195                 200                 205

Glu Phe Glu Arg Ser Lys Val Pro Asp Asp Arg Leu Tyr Glu Glu Leu
                210                 215                 220

His Val Tyr Ser Pro Ile Tyr Ser Ala Leu Glu Asp Thr Arg Glu Ala
            225                 230                 235

Ser Ala Pro Val Val Ser
        240
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 286 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
GTG AGA ACA TAT CTG TAATTGTTTC TGAAATGATG CTAACCAGAG ATTTTATTTT      55
Val Arg Thr Tyr Leu
  1               5

AATCAAAGAC AACTAATTTT CTTTTAATCA AGTGCTTATC TCTAGCCTTT CAATAATATC     115

TACAGTTCTT CATTTATATG CACATAGCCA TCTATAAATG TAGTTTCCAA AGCACTCTCT     175

ACATATACTC ATTAACAAGA GCAAATACAC TCACCACAGT TAACTATGGT TTAACCCATT     235

ACTATACTTT TATTGACTGA AAACCTTGAG ACTGTACAAA AAAAAAAAAA A              286
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Val Arg Thr Tyr Leu
 1               5
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 586 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(B) STRAIN: FcRI gamma subunit

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 23..76

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 77..283

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 23..283

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
AGCGCTGCAG CCCCCGCCCA GG ATG ATC CCA GCG GTG ATC TTG TTC TTG CTC        52
                         Met Ile Pro Ala Val Ile Leu Phe Leu Leu
                         -18         -15                 -10

CTT TTG GTG GAA GAA GCA GCT GCC CTA GGA GAG CCG CAG CTC TGC TAT       100
Leu Leu Val Glu Glu Ala Ala Ala Leu Gly Glu Pro Gln Leu Cys Tyr
            -5                   1               5

ATC CTG GAT GCC ATC CTG TTT TTG TAT GGT ATT GTC CTT ACC CTG CTC       148
Ile Leu Asp Ala Ile Leu Phe Leu Tyr Gly Ile Val Leu Thr Leu Leu
     10                  15                  20

TAC TGT CGA CTC AAG ATC CAG GTC CGA AAG GCA GAC ATA GCC AGC CGT       196
Tyr Cys Arg Leu Lys Ile Gln Val Arg Lys Ala Asp Ile Ala Ser Arg
 25                  30                  35                  40

GAG AAA TCA GAT GCT GTC TAC ACG GGC CTG AAC ACC CGG AAC CAG GAG       244
Glu Lys Ser Asp Ala Val Tyr Thr Gly Leu Asn Thr Arg Asn Gln Glu
                 45                  50                  55

ACA TAT GAG ACT CTG AAA CAT GAG AAA CCA CCC CAA TAGCTTTACA            290
Thr Tyr Glu Thr Leu Lys His Glu Lys Pro Pro Gln
            60                  65

ACACGTGTTC TCAGCTGCAT TCCTTTTCCG CTTTTAATTC TCTCCTCGCC CTCATGATTG     350
```

ACGTGGCTGT GCTACCTCCG TGCTTCTGGA ACTAGCTGAC CTTATTCCCA GAACCATGCT 410

AGGCTCTAAA TCAATGTCCC CATATCCACC AAAGACTTAC TCACTGACAT TTCTCTTCTC 470

CCATCCTCCT TTGCTTCATT CCTCTTTCCT TCCCTGATCC TCTGTGCTCA CTAAACAATG 530

GGAAGGGATT ACCCCCCAAT AAAGCTGCCA GAGATCACGC TCAAAAAAAA AAAAAA 586

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Ile Pro Ala Val Ile Leu Phe Leu Leu Leu Val Glu Glu Ala
-18 -15 -10 -5

Ala Ala Leu Gly Glu Pro Gln Leu Cys Tyr Ile Leu Asp Ala Ile Leu
1 5 10

Phe Leu Tyr Gly Ile Val Leu Thr Leu Leu Tyr Cys Arg Leu Lys Ile
15 20 25 30

Gln Val Arg Lys Ala Asp Ile Ala Ser Arg Glu Lys Ser Asp Ala Val
35 40 45

Tyr Thr Gly Leu Asn Thr Arg Asn Gln Glu Thr Tyr Glu Thr Leu Lys
50 55 60

His Glu Lys Pro Pro Gln
65

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 222 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: alpha subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Thr Gln Lys Ser Val Val Ser Leu Asp Pro Pro Trp Ile Arg Ile
1 5 10 15

Leu Thr Gly Asp Lys Val Thr Leu Ile Cys Asn Gly Asn Asn Ser Ser
20 25 30

Gln Met Asn Ser Thr Lys Trp Ile His Asn Asp Ser Ile Ser Asn Val
35 40 45

Lys Ser Ser His Trp Val Ile Val Ser Ala Thr Ile Gln Asp Ser Gly
50 55 60

```
Lys Tyr Ile Cys Gln Lys Gln Gly Phe Tyr Lys Ser Lys Pro Val Tyr
65                  70                  75                  80

Leu Asn Val Met Gln Glu Trp Leu Leu Leu Gln Ser Ser Ala Asp Val
                85                  90                  95

Val Leu Asp Asn Gly Ser Phe Asp Ile Arg Cys Arg Ser Trp Lys Lys
            100                 105                 110

Trp Lys Val His Lys Val Ile Tyr Tyr Lys Asp Asp Ile Ala Phe Lys
        115                 120                 125

Tyr Ser Tyr Asp Ser Asn Asn Ile Ser Ile Arg Lys Ala Thr Phe Asn
    130                 135                 140

Asp Ser Gly Ser Tyr His Cys Thr Gly Tyr Leu Asn Lys Val Glu Cys
145                 150                 155                 160

Lys Ser Asp Lys Phe Ser Ile Ala Val Val Lys Asp Tyr Thr Ile Glu
                165                 170                 175

Tyr Arg Trp Leu Gln Leu Ile Phe Pro Ser Leu Ala Val Ile Leu Phe
            180                 185                 190

Ala Val Asp Thr Gly Leu Trp Phe Ser Thr His Lys Gln Phe Glu Ser
        195                 200                 205

Ile Leu Lys Ile Gln Lys Thr Gly Lys Gly Lys Lys Lys Gly
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 243 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: beta subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Met Asp Thr Glu Asn Lys Ser Arg Ala Asp Leu Ala Leu Pro Asn Pro
1               5                   10                  15

Gln Glu Ser Pro Ser Ala Pro Asp Ile Glu Leu Leu Glu Ala Ser Pro
            20                  25                  30

Pro Ala Lys Ala Leu Pro Glu Lys Pro Ala Ser Pro Pro Pro Gln Gln
        35                  40                  45

Thr Trp Gln Ser Phe Leu Lys Lys Glu Leu Glu Phe Leu Gly Val Thr
    50                  55                  60

Gln Val Leu Val Gly Leu Ile Cys Leu Cys Phe Gly Thr Val Val Cys
65                  70                  75                  80

Ser Thr Leu Gln Thr Ser Asp Phe Asp Asp Glu Val Leu Leu Leu Tyr
                85                  90                  95

Arg Ala Gly Tyr Pro Phe Trp Gly Ala Val Leu Phe Val Leu Ser Gly
            100                 105                 110
```

```
Phe Leu Ser Ile Met Ser Glu Arg Lys Asn Thr Leu Tyr Leu Val Arg
        115                 120                 125

Gly Ser Leu Gly Ala Asn Ile Val Ser Ser Ile Ala Ala Gly Leu Gly
    130                 135                 140

Ile Ala Ile Leu Ile Leu Asn Leu Ser Asn Asn Ser Ala Tyr Met Asn
145                 150                 155                 160

Tyr Cys Lys Asp Ile Thr Glu Asp Asp Gly Cys Phe Val Thr Ser Phe
                165                 170                 175

Ile Thr Glu Leu Val Leu Met Leu Leu Phe Leu Thr Ile Leu Ala Phe
            180                 185                 190

Cys Ser Ala Val Leu Leu Ile Ile Tyr Arg Ile Gly Gln Glu Phe Glu
        195                 200                 205

Arg Ser Lys Val Pro Asp Asp Arg Leu Tyr Glu Glu Leu His Val Tyr
    210                 215                 220

Ser Pro Ile Tyr Ser Ala Leu Glu Asp Thr Arg Glu Ala Ser Ala Pro
225                 230                 235                 240

Val Val Ser
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(B) STRAIN: gamma subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Leu Gly Glu Pro Gln Leu Cys Tyr Ile Leu Asp Ala Ile Leu Phe Leu
1               5                   10                  15

Tyr Gly Ile Val Leu Thr Leu Leu Tyr Cys Arg Leu Lys Ile Gln Val
            20                  25                  30

Arg Lys Ala Asp Ile Ala Ser Arg Glu Lys Ser Asp Ala Val Tyr Thr
        35                  40                  45

Gly Leu Asn Thr Arg Asn Gln Glu Thr Tyr Glu Thr Leu Lys
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11298 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:
(A) ORGANISM: homo sapien
(B) STRAIN: FcRI beta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAGCTTTTCA AAGGTGCAAT TGGATAACTT CTGCCATGAG AAATGGCTGA ATTGGGACAC 60

AAGTGGGGAC AATTCCAGAA GAAGGGCACA TCTCTTTCTT TTCTGCAGTT CTTTCTCACC 120

TTCTCAACTC CTACTAAAAT GTCTCATTTT CAGGTTCTGT AAATCCTGCT AGTCTCAGGC 180

AAAATTATGC TCCAGGAGTC TCAAATTTTC TTATTTCATA TTAGTCTTTA TTTAGTAGAC 240

TTCTCAATTT TTCTATTCAT CACAAGTAAA AGCCTGTTGA TCTTAATCAG CCAAGAAACT 300

TATCTGTCTG GCAAATGACT TATGTATAAA GAGAATCATC AATGTCATGA GGTAACCCAT 360

TTCAACTGCC TATTCAGAGC ATGCAGTAAG AGGAAATCCA CCAAGTCTCA ATATAATAAT 420

ATTCTTTATT CCTGGACAGC TCGGTTAATG AAAAAATGGA CACAGAAAGT AATAGGAGAG 480

CAAATCTTGC TCTCCCACAG GAGCCTTCCA GGTAGGTACA AGGTATTATT TTTTTCTACC 540

CTCAGTCACT TGTGGCAGGG GAAGTCATAG TCACGGTGCT TAGGAGATGA AACTTTATTG 600

ATTTAGGCAT GGATCCATCT AGTTTAATTA ATATATTGGG TATGAGGAAG CTACTTGCTG 660

TACTTTCCAT GTGGTTCTCT CTCCCTGGAG AGGAACATTT TTACTCAGCT TGCAAACTGG 720

AAATAGATTT TCTCACATTA GAAGCTCATT TTCTGGGTAT GAGACAGGAG AGTTCATACT 780

GTGTATGTAG ATCTCTGGCT TCTGGGTCTG ACATGTGCTG AGGGACACAT ATCCTTCACA 840

CATGCTTTTA TAAATACTTG ATAAAGTAAC CTGCTTCTTG ATTGGTCTTT ATAATCCATA 900

AGCTGTGGGA TGCTTCTCTG AAGATGAAAA TAGTAATAGA GTCCCATCTA GCTATTCAAA 960

GCCATTCCTT CATTGTATTC TGTGCACATG AAGTTGGGGT TTGTTACTGA CAAAATATAT 1020

TCAGATACAT TTCTATGTTA AAAGGATTGT GAGATGCATA GGTAAATGTG TTTATTTTCA 1080

GTTTTACTTG TCAACATAGA TGAATGAGAA AGAACTTGAA AGTAACACTG GATTAAGAAT 1140

AGGAAAATTT GGCATGGATT TTGCTCCATT TTGTCCCATC TAATCACTTG GATAGTGTTC 1200

AGGTGTTCTT GGTCAGTTAC TTGGATGCTC TGAGCTTTAG TTTCTTGGTG ATTACAATGA 1260

AGATTTGAAT TACAGGATGG CTTTGAAAAA ATAAACAAAA CTCCCCTTTC TGTCTGTCGA 1320

GAATGTTGCA CAGGGAGTTA CAGAATGTTC TCATGACTGA ATTGCTTTTA AATTTCACAG 1380

TGTGCCTGCA TTTGAAGTCT TGGAAATATC TCCCCAGGAA GTATCTTCAG GCAGACTATT 1440

GAAGTCGGCC TCATCCCCAC CACTGCATAC ATGGCTGACA GTTTTGAAAA AAGAGCAGGA 1500

GTTCCTGGCG GTGAGTGAGC CTCCTCCAAC TTTGACTAGA GTAAGGGTTG GGTCTAGAAA 1560

AGAATATTGA GTTGCATCAA CTGTTTTCCC ACTTGGATTC ATGAGAGGTG TTAGGTCCTT 1620

TAAAAAACAT GGTAGATAAA GAGTTGACAC TAACTGGGTC CTTTTGGGAA GAGCCAGAAG 1680

CATTTCCTCA TAAAGACTTT AAATTGCTAG GACGAGAATG GCCAACAGGA GTGAAGGATT 1740

CATAACTTTA TCTTTACTTA GATGTAAAGA ACAATTACTG ATGTTCAACA TGACTACATA 1800

CATAAAGGCG CATGGAGAAA AGTATTGGCC TTCCATGCAT TAGGTAGTGC TTGTATCAAT 1860

TCTTATAGTG GCTAGGGTAT CCTGGAAAAT CTTACGTGTG GATCATTTCT CAGGACAGTC 1920

TAGGACACTA ACGCAGTTTC TCATGTTTGG CTTCTATTAT TAAAAAATGA TACAATCTCG 1980

GGAAAATTTT TTTGATTTTC ATGAAATTCA TGTGTTTTTC TATAGGTAAC ACAAATTCTG 2040

ACTGCTATGA TATGCCTTTG TTTTGGAACA GTTGTCTGCT CTGTACTTGA TATTTCACAC 2100

ATTGAGGGAG ACATTTTTTC ATCATTTAAA GCAGGTTATC CATTCTGGGG AGCCATATTT 2160

GTGAGTATAT ATCTATAATT GTTTCTGAAA TAACACTGAA CATAGGTTTT TCTCTTTCTC 2220

AGATCTAACC AGTTGTTTAT TCCCAGTATT AAGATGATAT TTATAATTCT TAATTATAAA 2280

TATATGTGAG CATATATAAC ATAGATATGC TCATTAACAA CAACAAAAGA TTCTTTTTAC 2340

AATTAACGGT GGGTTAAACA TTTAGCCCAC AGTTTTATCC CATGAGAAAC CTGAATCTAA 2400

TACAAGTTAA ATGACTTGCC TAAGGGCCAC TTGACTAATA GTAATTGAAC CTAAACTTTC 2460

AGAATCCAAC TCCAGGAACA TACTTCTAGC ACTATTCATC AATAAAGTTA TATGATAAAT 2520

ACATACAACT TTATCTGTCA ACTAAAAATA ACAACAGAGG CTGGGCATGG TGGCTCACAC 2580

CCGTAATCCC AGCACTTTGG GAGGCTGAGG CAGGTGGATC ACCTGAGGTC AGGAGTTTGA 2640

GACCAGCCTG ACCAACATGG TGAAACCTCA TCTCTACTAA ATATAAAAAA TTAGCTGAGT 2700

GTGATAGTGC ATACCTGTAA TCCAGCTACT TAAGAGGCTG AGGCAGGAGG CTTGTTTGAA 2760

CCTGGAAGGC AGAGGTTGCA GTGAGCTGAG ATTGTGCCAT TGCACTCCAG CCTGGGCAAT 2820

AAGTGCGAAC TCTGTCTCAA AATAATAATA ATAATAATAG AAAATAAAGT TGTCTTCATG 2880

AAAAATGAGG AAAGAGATTG CTGGGGTGAG AAACATTAAG ATCAATGGGC ATATGGTGAC 2940

CTTCTATGCC CTAGAAACTC TTTTANGGTA TTTTCTCCTG GTATCTCTTT TACNCATCGT 3000

TCTATCTGGA AAAATAGGTG GATGAGTGAG ATAATAACGG TATATACTTT TTAAAGGTCT 3060

AATTGACATA TATAAATTGC AAGTATTTCA GATGTCAATT TGCTAACCTT GACACACATA 3120

GACACACATG AAAACATCAC CACATTAATA CAATGTATGT ATCCATCATT CCAAAAGCTT 3180

CCCTGTGTAT CTTTGTAACT CTTTCTTCCT CCCTCCACTC CTTGTCCTCT CGTTCCCAAG 3240

AAAACATTGA TCTGCTTCCT GTGAATATAA ATTAACTTAC ATTTTTTAGA GCTTTATATA 3300

AGTATGTTCT CTTTACTGTT TGTCTTCCTT CGCTGCACAG TTATTTTGAG ATTCTTCAAG 3360

TTTTTTCTTT ATATCGATAC TTCATTCACA AGAATATATT TTAATTCTAG ACTATGTCAC 3420

ATTGACTTTG TCGTCTGCTA AATCCTTAGT GCTCAGATGA CTTGTTCAGG ACTCTCCTTG 3480

AACCTGTACC TCTGTTANAT TGAAACTTGT CTCTACTGTC TTTTTATTTC AAACACAGCT 3540

TATTAGGTGT CTCTCAACCC ATCAAACNCA CAATCTGAGT CTTTAGGAGA TTGCTTTGAA 3600

TTTGTGCTAT TGACTTATAT NTATATNAAA TNTGTAAATG TTTGGTAAAA ATATCATCAT 3660

GTACNTTTTC ATAATTACGC TATNTNCACA TGATATATGT CAGACTCTGG AAATATGCAT 3720

GCCACAGACA CGTGTTTCTT GCCTAAAGGG GCTGATGGAA GACNCACATA CNAATAGACG 3780

ATTGCAGTAG AATGAGAGTG GTGGTCTAAN CAGTACATGT CCTGATGTTG CTCGGACAGT 3840

TACTACNCCA AGAGTACCCC CTGCATTGTC AGGGTTAGCA TCTCCTGGAA GCCTCATGTA 3900

AATGAAGAAT TTCATGCTCC ATCCAGGACC TAATGAATAA GAATCTGCAT TTTAGCAAGA 3960

CCCTCATATG ATTCATATAC ACTTTTTTTT TTTTTTTTTA GATGGAGTCT CACTCTTGTC 4020

GCCCAGGCTG GAGTGCAATG GCATGATCTT GGCTCACTGC AACCTCTGCC TCCCGGGTTC 4080

AAGTGATTCT CCTGTCTCAG CCTCCCTAGT AGCTGGGACT ACAGGTGCAT GCCACAGTGG 4140

CTGGCTAATT TTTGTATTTT TAGTAGAGAC AGGGTTTCAC CATTTTGGTC AGGCTGGTCT 4200

TGAACTCATG ACCTCCGGTG ATTCCCCCGC CTCGGCTTCC CAAAGTGCTG GGATTACAGA 4260

CATGAGCCAC CACACCCGCC TTATTCGTAT ACNCATTTAA TTCTGAGAAG CACTCTATAG 4320

AAAATAAGAA TAAGAAAATA TTGGGCTCAC AGGTGACATT AATAAGTAAC TTTATCGAGT 4380

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TCATTGTGGC TTTTTTTTCC TCCTTTTTGA ACAGTTTTCT ATTTCTGGAA TGTTGTCAAT 4500

TATATCTGAA AGGAGAAATG CAACATATCT GGTGAGTTGC CCGTTTCTGT CTTTGTCCAT 4560

CCTTGAAAAG ATAAGAAGAA CAGAGTTTTA AGAGTCTTAA GGGAAACACA TCTTTGTCTC 4620

CTATATTACT TGTGAATGTG GATATATGAT TTTGTTTCAA TCTATTTTGT GTCCTAAGGC 4680

TTTTTGCAAC AGAAGTTGGA TATATCATTA GAAACATAAA TTGTACCATT TAACATACAT 4740

GAAGTTTATG TTTACCTTGA CGTTCTTCTA AAAAGTGTCC TACACCGGCA TTGTCCTTGT 4800

AGGCATATTC ACATGATCAA ATAAAATAAT TAGTTTTCAA TTAAGGAGAA TATTTGAGGA 4860

AAGACCGTAC GTGTTCATGT GGTTCCTGAA GGCAGTCCAG TGAGAAAGTA ATATATGCTT 4920

CATTAAACAA TGCGGACATT TTCAGGGTTT CCCTTTTTAA CCAAAATTTG GAAGCAATGT 4980

GGAATTTACT GGATGCATCC AGCCCTGAAA TGAAGATAGG TTTATTGAAT GTGCCAGCAA 5040

GTGCAGGCCC AGGTCTGAGT GTTCTTCATT ATTATCAGGT GAGAGGAAGC CTGGGAGCAA 5100

ACACTGCCAG CAGCATAGCT GGGGGAACGG GAATTACCAT CCTGATCATC AACCTGAAGA 5160

AGAGCTTGGC CTATATCCAC ATCCACAGTT GCCAGAAATT TTTTGAGACC AAGTGCTTTA 5220

TGGCTTCCTT TTCCACTGTA TGTATTTTTT TTTGTGTGGG AAGACTAAGA TTCTGGGTCC 5280

TAATGTAAGT AAGAAGCCCT CTTCTCCTGT TCCATGAACA CCATCCTTTT CTGTAACTTC 5340

TATTACACAG TATAGTGGTT CTGTAAGTTC ACACAGCCCA GGGAGATGCT GGCTGCCCAC 5400

TCCCCTCAAC CCAGGCAAAT TCCTCGGGGT TAAAGTTATC TACTGCAAGT GACGATCTCT 5460

GGGTTTTTCT GTGCCTGTGT TTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTATGTGTCA 5520

CTTTAAAAGG ACTGGTCAGA TGGTAGGGAG ATGAAAACAG GAGATGCTAT AAGAAAATAA 5580

ACTTTTGGGG CGAATACCAA TGTGACTCTT TTTGTTTGTC ATTTGTTGCT GTTCAATAGG 5640

AAATTGTAGT GATGATGCTG TTTCTCACCA TTCTGGGACT TGGTAGTGCT GTGTCACTCA 5700

CAATCTGTGG AGCTGGGGAA GAACTCAAAG GAAACAAGGT AGATAGAAGC CCGATATAAA 5760

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ACTTGCTCTA AAACCTCAGT TTTCTTTCCT TTCTGATTCA TGACTACCAA ATGTTTTCAC 6180

TTGCCTCACC CGTCCATTAC ACCTTTGATA AGAACCACCA GACCTTGTGC TCATGTACTT 6240

GCCCATGTCT GATGGAAGAA ACATACTCTC TCCATCTGTC CACTTTCCTG AGGCATTCAA 6300

GTCTAGCCAC CTTTTAAAAT CACTCTCCTC CAGGCTGGGC ACGGTGTCAC GCCTGTAATC 6360

TCAGCACTTT GTGAGGCTGA GGAGGGCGGA TCACTTGAAG TCAGGAGTTC AAAACCAGCC 6420

TGGCCAAATG GCAAAACCAA ATCTTCTTCA ATTATAACCA AATCTTAAAC CAAATCTCTA 6480

CTAAAAAATA CAACAAAACA AAACAACAAC AACAAAAACA GAAAAGGAAA CATTAGCCCA 6540

GCGTGGTGGC AGGTACCTGA GGTTCCAGAT ACTTGGGAGG CTGAAGCAGG AGAATCGCTT 6600

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TGAAATGATT TTTCCCTTAT CAGGTTCCAG AGGATCGTGT TTATGAAGAA TTAAACATAT 7260

ATTCAGCTAC TTACAGTGAG TTGGAAGACC CAGGGGAAAT GTCTCCTCCC ATTGATTTAT 7320

AAGAATCACG TGTCCAGAAC ACTCTGATTC ACAGCCAAGG ATCCAGAAGG CCAAGGTTTT 7380

GTTAAGGGGC TACTGGAAAA ATTTCTATTC TCTCCACAGC CTGCTGGTTT TACATTAGAT 7440

TTATTCGCCT GATAAGAATA TTTTGTTTCT GCTGCTTCTG TCCACCTTAA TATGCTCCTT 7500

CTATTTGTAG ATATGATAGA CTCCTATTTT TCTTGTTTTA TATTATGACC ACACACATCT 7560

CTGCTGGAAA GTCAACATGT AGTAAGCAAG ATTTAACTGT TTGATTATAA CTGTGCAAAT 7620

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GTTCTTAGGG TTTTTTTTTT TTTTAGCATT CTTAATAGTT ACAGTTGGGC ATGATTTGTA 7740

CCATCCACCC ATACCCACAC AGTCACAGTC ACACACACAT ATGTATTACT TACACTATAT 7800

ATAACTTCCT ATGCAAATAT TTTACCACCA GTCAATAATA CATTTTTGCC AAGACATGAA 7860

GTTTTATAAA GATCTGTATA ATTGCCTGAA TCACCAGCAC ATTCACTGAC ATGATATTAT 7920

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GAACTAGCCG CCTATTCCTG TTTCTGTTTT ATTCCTTTGT TTCTTGACTT TTCCTTTCCA 6120

ACTTGCTCTA AAACCTCAGT TTTCTTTCCT TTCTGATTCA TGACTACCAA ATGTTTTCAC 6180

TTGCCTCACC CGTCCATTAC ACCTTTGATA AGAACCACCA GACCTTGTGC TCATGTACTT 6240

GCCCATGTCT GATGGAAGAA ACATACTCTC TCCATCTGTC CACTTTCCTG AGGCATTCAA 6300

GTCTAGCCAC CTTTTAAAAT CACTCTCCTC CAGGCTGGGC ACGGTGTCAC GCCTGTAATC 6360

TCAGCACTTT GTGAGGCTGA GGAGGGCGGA TCACTTGAAG TCAGGAGTTC AAAACCAGCC 6420

TGGCCAAATG GCAAAACCAA ATCTTCTTCA ATTATAACCA AATCTTAAAC CAAATCTCTA 6480

CTAAAAAATA CAACAAAACA AAACAACAAC AACAAAAACA GAAAAGGAAA CATTAGCCCA 6540

GCGTGGTGGC AGGTACCTGA GGTTCCAGAT ACTTGGGAGG CTGAAGCAGG AGAATCGCTT 6600

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GACAGAGCCA TACTTCCCAG CACATTGGGA GGCCAAAGCT GAAGAATAAT TTGAGGTGAG 6720

GATTTGGAGA CCAGCCTGGC CAACATGGTG AAACTCCGTC TGTACTAAAA ATATAAAACT 6780

TAGTGGGGCA TGGGGGCACA CACCTGTAAT TTCAGCTACT TAGGAGGCTG AGGCAGGAGA 6840

ATTGCTTGAA CCCGGGAGGC GGAAGTTGCA GTGAGCCAAG ATCGTGGCCA CTGCACTCCA 6900

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TCATTGACTG ACATGATTTC TTTTGGCCCA CTTGGCCAGC TAGTCTGGTT TGGTTTTCTG 7080

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TAGATGAGGT AAGTCTCTTG AGCGAGACTT CTAGGGATGG GAAATTTGTG GTGATTGAT` 7200

TGAAATGATT TTTCCCTTAT CAGGTTCCAG AGGATCGTGT TTATGAAGAA TTAAACATAT 7260

ATTCAGCTAC TTACAGTGAG TTGGAAGACC CAGGGGAAAT GTCTCCTCCC ATTGATTTAT 7320

AAGAATCACG TGTCCAGAAC ACTCTGATTC ACAGCCAAGG ATCCAGAAGG CCAAGGTTTT 7380

GTTAAGGGGC TACTGGAAAA ATTTCTATTC TCTCCACAGC CTGCTGGTTT TACATTAGAT 7440

TTATTCGCCT GATAAGAATA TTTTGTTTCT GCTGCTTCTG TCCACCTTAA TATGCTCCTT 7500

CTATTTGTAG ATATGATAGA CTCCTATTTT TCTTGTTTTA TATTATGACC ACACACATCT 7560

CTGCTGGAAA GTCAACATGT AGTAAGCAAG ATTTAACTGT TTGATTATAA CTGTGCAAAT 7620

ACAGAAAAAA AGAAGGCTGG CTGAAAGTTG AGTTAAACTT TGACAGTTTG ATAATATTTG 7680

GTTCTTAGGG TTTTTTTTTT TTTTAGCATT CTTAATAGTT ACAGTTGGGC ATGATTTGTA 7740

CCATCCACCC ATACCCACAC AGTCACAGTC ACACACACAT ATGTATTACT TACACTATAT 7800

ATAACTTCCT ATGCAAATAT TTTACCACCA GTCAATAATA CATTTTTGCC AAGACATGAA 7860

GTTTTATAAA GATCTGTATA ATTGCCTGAA TCACCAGCAC ATTCACTGAC ATGATATTAT 7920

TTGCAGATTG ACAAGTAGGA AGTGGGGAAC TTTTATTAAG TTACTCGTTG TCTGGGGAGG 7980

TAAATAGGTT AAAAACAGGG AAATTATAAG TGCAGAGATT AACATTTCAC AAATGTTTAG 8040

TGAAACATTT GTGAAAAAAG AAGACTAAAT TAAGACCTGA GCTGAAATAA AGTGACGTGG 8100

AAATGGAAAT AATGGTTATA TCTAAAACAT GTAGAAAAAG AGTAACTGGT AGATTTTGTT 8160

AACAAATTAA AGAATAAAGT TAGACAAGCA ACTGGTTGAC TAATACATTA AGCGTTTGAG 8220

TCTAAGATGA AAGGAGAACA CTGGTTATGT TGATAGAATG ATAAAAAGGG TCGGGCGCGG 8280

AGGCTCACGC CTGTAATCCC AGCCCTTTGG GAGGCCGAGG TGGGCAGATC ACGAAGTCAG 8340

TAGTTTGAGA CCAGCCTGGC CAACATAGTG AAACCCCGTC TCTACTAAAA ATACAAAAAA 8400

AAAATTAGCT GGGTGTGGTG GCAGTCACCT GTAGTCCCAG CTACTTGGGA GGATGAGGCA 8460

GGAGAATCGC TTGAACCTGG GAGGCGGAGG TTGCAGTGAG CCGAGATCGC ACCAGTGCAC 8520

TCCAGCCTTG GTGACAATGG GAGACTCCAT CTCAAAAAAA AAAAAAAAAA AAAAAAGATA 8580

AAAAGTCAGA AATCTGAAAA GTGGAGGAAG AGTACAAATA GACCTAAATT AAGTCTCATT 8640

TTTTGGCTTT GATTTTGGGG AGACAAAGGG AAATGCAGCC ATAGAGGGCC TGATGACATC 8700

CAATACATGA GTTCTGGTAA AGATAAAATT TGATACACGG TTTGGTGTCA TTATAAGAGA 8760

AATCATTATT AAATGAAGCA AGTTAACACT CTAAGAGAAT TATTTTGAGA TAGAAGTGAA 8820

GCTAAGCTAA ACTTCACATG CCTATAATTG GAGGGAAAAA CTAAGGATAA AATCTAGCCT 8880

AGAAGATACA ATAATTAGTC ATAAACATGC ATTGTGAAAC TGTAGAGAGC AGGTAGCCCA 8940

AAATAGAGAA AGATTAGATA AAGAGAAAAT AAGTATCCAT CAGAGACAGT ATCTCTAGGC 9000

TTGGGCAAGA GAAAAGTCCA CAGTGATAAG CAACTCCACC TAAGGCATGA ATATGCGGCA 9060

GAGAAAACAG CAATAGTGAA TGAATGCAAA AGGTGCTGAG CAAATTCCAC ACATGAGTAT 9120

TGTGCATGAG TAAATGAATA AAACATTTGC AAAGACCTTT AGAGAAAGAG AATGGGAGCA 9180

TATGTGCGAA ATAAGATAGT TGATTATGAA TAGAAGGTAG TGAAGAAAAG CAAGCTAAGA 9240

AAAAATTCTG TTTATAAAAG AAGGAAAAGA TAGTTTATGT TTTTAGCCTA AGTATAAGAG 9300

TCCTACAGAT GGACTGAAAA AAATCAGTCT GAGAGTATTA GTCACAATTA ATGAAATAAT 9360

TACATTTTAT GTATTGAGGA TGCCAAGATT AAAAGGTGAC AGGTAGATGT TAATTTCCCT 9420

AGATTGTGAA AGTGATCACG ACAATCACAC AACAAATAAT TAAGTGACTT GGTATGCTTT 9480

ATTTAATTGT AGGGCCTGAG GTTTTCCATT CTCATTTTTC TAAAATACAA TTTTGTTTCT 9540

CCAAATTTGA CAGCAGAATA AAAACCCTAC CCTTTCACTG TGTATCATGC TAAGCTGCAT 9600

CTCTACTCTT GATCATCTGT AGGTATTAAT CACATCACTT CCATGGCATG GATGTTCACA 9660

TACAGACTCT TAACCCTGGT ATACCAGGAC CTCTAGGAGT GGATCCAATC TATATCTTTA 9720

CAGTTGTATA GTATATGATA TCTCTTTTAT TTCACTCAAT TTATATTTTC ATCATTGACT 9780

ACATATTTCT TATACACAAC ACACAATTTA TGAATTTTTT CTCAAGATCA TTCTGAGAGT 9840

TGCCCCACCC TACCTGCCTT TTATAGTACG CCCACCTCAG GCAGACACAG AGCACAATGC 9900

TGGGGTTCTC TTCACACTAT CACTGCCCCA AATTGTCTTT CTAAATTTCA ACTTCAATGT 9960

CATCTTCTCC ATGAAGACCA CTGAATGAAC ACCTTTTCAT CCAGCCTTAA TTTCTTGCTC 10020

CATAACTACT CTATCCCACG ATGCAGTATT GTATCATTAA TTATTAGTGT GCTTGTGACC 10080

TCCTTATGTA TTCTCAATTA CCTGTATTTG TGCAATAAAT TGGAATAATG TAACTTGATT 10140

TCTTATCTGT GTTTGTGTTG GCATGCAAGA TTTAGGTACT TATCAAGATA ATGGGGAATT 10200

AAGGCATCAA TAAAATGATG CCAAAGACCA AGAGCAGTTT CTGAAGTCCT CCTTTTCATC 10260

AGCTCTTTAT CAAACAGAAC ACTCTATAAA CAACCCATAG CCAGAAAACA GGATGTAGGA 10320

ACAATCACCA GCACACTCTA TAAACAACCC ATAGCCAGAA AACAGAATGT AAGGACAATC 10380

ACCAGCCATC TTTTGTCAAT AATTGATGGA ATAGAGTTGA AAGGAACTGG AGCATGAGTC 10440

ATATTTGACC AGTCAGTCCT CACTCTTATT TACTTGCTAT GTAAACTTGA GAAAGCTTTT 10500

TTCTCTTTGT GAACCTCAGG TTTTACATCT GAAAATGAGA AATTTGGAAC AAAAGATTCC 10560

TAACTGGTCT TTCTGTTCCC ATATTCTGTG ATTTTTCAAT ATTTAGGATT TTTGGTAATC 10620

ACAATTACTT AGTTTGTGGT TGAGATAGCA ACACGAATCA GAACTATTTG GTGGACATAT 10680

TTTCAAAGGA GTAGCTCTCC ACTTTGGGTA AAGAAGTGAT GCNGGTCGTG GTGGCTCACG 10740

CCTGTAATCC CAGCACTTTA GGGAGGCCAA GGCGGGTGGA TCACGAGGTC AGGAGATCGA 10800

GACCATCCTG GCTAACACGG TGAAACCCCG TCTCTACTAA AAAATACAAA AAATTAGCCA 10860

GGCGTGGTGG CGGGCGCCTG TAGTCCCACG TACTCGGGAG GCTGAGGCAG GAGAATGGCA 10920

TGAACCAGGG AGGCGGAGCT TGCCGTGAGC CGAGATAGCG CCACTGCAGT CCCTCCTGGG 10980

CAAAAGAGCA AGACTGCGTC TCAAAAAAAA AAAAAAAAAA AAAAAAAGAA GTGTGTGGAG 11040

TAGCAGGACA CCTGCAACAA TAATATTTTT CTAAATCCCT CTGAAAAATG CTAATCAAAG 11100

GGTTTTTTTC CTAAAAATTG TCTTAGAAAT AAAATTTCCC CTTTGGGAGA CCGAGGCTGG 11160

CAGATCACGA GGTCAGGAGA TAGAGACCAC GGTGAAACCC CGTCTCTACT AAAAATACTA 11220

AAAATTAGCC GGGGNGTGGT GGTGGGTACA CCTGTAGTCC CAGCTACTTG GAGGCTGAGG 11280

CTGGAGAATC ACGTGAAC 11298

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 244 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: homo sapien
(B) STRAIN: FcRI beta subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Asp Thr Glu Ser Asn Arg Arg Ala Asn Leu Ala Leu Pro Gln Glu
1 5 10 15

Pro Ser Ser Val Pro Ala Phe Glu Val Leu Glu Ile Ser Pro Gln Glu
20 25 30

Val Ser Ser Gly Arg Leu Leu Lys Ser Ala Ser Ser Pro Pro Leu His
35 40 45

Thr Trp Leu Thr Val Leu Lys Lys Glu Gln Glu Phe Leu Gly Val Thr
50 55 60

Gln Ile Leu Thr Ala Met Ile Cys Leu Cys Phe Gly Thr Val Val Cys
65 70 75 80

Ser Val Leu Asp Ile Ser His Ile Glu Gly Asp Ile Phe Ser Ser Phe
85 90 95

Lys Ala Gly Tyr Pro Phe Trp Gly Ala Ile Phe Phe Ser Ile Ser Gly
100 105 110

Met Leu Ser Ile Ile Ser Glu Arg Arg Asn Ala Thr Tyr Leu Val Arg
115 120 125

Gly Ser Leu Gly Ala Asn Thr Ala Ser Ser Ile Ala Gly Gly Thr Gly
130 135 140

Ile Thr Ile Leu Ile Ile Asn Leu Lys Lys Ser Leu Ala Tyr Ile His
145 150 155 160

Ile His Ser Cys Gln Lys Phe Phe Glu Thr Lys Cys Phe Met Ala Ser
165 170 175

Phe Ser Thr Glu Ile Val Val Met Met Leu Phe Leu Thr Ile Leu Gly
180 185 190

Leu Gly Ser Ala Val Ser Leu Thr Ile Cys Gly Ala Gly Glu Glu Leu
195 200 205

Lys Gly Asn Lys Val Pro Glu Asp Arg Val Tyr Glu Glu Leu Asn Ile
210 215 220

Tyr Ser Ala Thr Tyr Ser Glu Leu Glu Asp Pro Gly Glu Met Ser Pro
225 230 235 240

Pro Ile Asp Leu

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 243 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(B) STRAIN: FcRI beta subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Asp Thr Glu Asn Lys Ser Arg Ala Asp Leu Ala Leu Pro Asn Pro
1 5 10 15

Gln Glu Ser Pro Ser Ala Pro Asp Ile Glu Leu Leu Glu Ala Ser Pro
20 25 30

Pro Ala Lys Ala Leu Pro Glu Lys Pro Ala Ser Pro Pro Pro Gln Gln
35 40 45

Thr Trp Gln Ser Phe Leu Lys Lys Glu Leu Glu Phe Leu Gly Val Thr
50 55 60

Gln Val Leu Val Gly Leu Ile Cys Leu Cys Phe Gly Thr Val Val Cys
65 70 75 80

```
Ser Thr Leu Gln Thr Ser Asp Phe Asp Asp Glu Val Leu Leu Leu Tyr
                85                  90                  95

Arg Ala Gly Tyr Pro Phe Trp Gly Ala Val Leu Phe Val Leu Ser Gly
            100                 105                 110

Phe Leu Ser Ile Met Ser Glu Arg Lys Asn Thr Leu Tyr Leu Val Arg
        115                 120                 125

Gly Ser Leu Gly Ala Asn Ile Val Ser Ser Ile Ala Ala Gly Leu Gly
    130                 135                 140

Ile Ala Ile Leu Ile Leu Asn Leu Ser Asn Asn Ser Ala Tyr Met Asn
145                 150                 155                 160

Tyr Cys Lys Asp Ile Thr Glu Asp Asp Gly Cys Phe Val Thr Ser Phe
                165                 170                 175

Ile Thr Glu Leu Val Leu Met Leu Leu Phe Leu Thr Ile Leu Ala Phe
            180                 185                 190

Cys Ser Ala Val Leu Leu Ile Ile Tyr Arg Ile Gly Gln Glu Phe Glu
        195                 200                 205

Arg Ser Lys Val Pro Asp Asp Arg Leu Tyr Glu Glu Leu His Val Tyr
    210                 215                 220

Ser Pro Ile Tyr Ser Ala Leu Glu Asp Thr Arg Glu Ala Ser Ala Pro
225                 230                 235                 240

Val Val Ser
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 235 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: FcRI.beta subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Met Asp Thr Glu Asn Arg Ser Arg Ala Asp Leu Ala Leu Pro Asn Pro
1               5                   10                  15

Gln Glu Ser Ser Ser Ala Pro Asp Ile Glu Leu Leu Glu Ala Ser Pro
            20                  25                  30

Ala Lys Ala Ala Pro Pro Lys Gln Thr Trp Arg Thr Phe Leu Lys Lys
        35                  40                  45

Glu Leu Glu Phe Leu Gly Ala Thr Gln Ile Leu Val Gly Leu Ile Cys
    50                  55                  60

Leu Cys Phe Gly Thr Ile Val Cys Ser Val Leu Tyr Val Ser Asp Phe
65                  70                  75                  80

Asp Glu Glu Val Leu Leu Leu Tyr Lys Leu Gly Tyr Pro Phe Trp Gly
                85                  90                  95
```

Ala Val Leu Phe Val Leu Ser Gly Phe Leu Ser Ile Ile Ser Glu Arg
100 105 110

Lys Asn Thr Leu Tyr Leu Val Arg Gly Ser Leu Gly Ala Asn Ile Val
115 120 125

Ser Ser Ile Ala Ala Gly Thr Gly Ile Ala Met Leu Ile Leu Asn Leu
130 135 140

Thr Asn Asn Phe Ala Tyr Met Asn Asn Cys Lys Asn Val Thr Glu Asp
145 150 155 160

Asp Gly Cys Phe Val Ala Ser Phe Thr Thr Glu Leu Val Leu Met Met
165 170 175

Leu Phe Leu Thr Ile Leu Ala Phe Cys Ser Ala Val Leu Phe Thr Ile
180 185 190

Tyr Arg Ile Gly Gln Glu Leu Glu Ser Lys Lys Val Pro Asp Asp Arg
195 200 205

Leu Tyr Glu Glu Leu Asn Val Tyr Ser Pro Ile Tyr Ser Glu Leu Glu
210 215 220

Asp Lys Gly Glu Thr Ser Ser Pro Val Asp Ser
225 230 235

WHAT IS CLAIMED IS:

1. A nucleic acid sequence capable of coding for a polypeptide having an amino acid sequence corresponding to the human beta subunit of FcεRI.

2. The nucleic acid sequence of claim 1 further defined as a DNA sequence.

3. The DNA sequence according to claim 2, wherein said DNA sequence corresponds to that shown in FIG. 14 (SEQ ID NO:31), or an allelic or species variation thereof.

4. The DNA sequence according to claim 2, wherein said DNA segment encodes the human amino acid sequence set forth in FIG. 19 (SEQ ID NO:32), or an allelic or species variation thereof.

5. A polypeptide having an amino acid sequence corresponding to the human beta subunit of FcεRI, isolated from its natural environment.

6. The polypeptide according to claim 5, wherein said polypeptide has the human amino acid sequence set forth in FIG. 19 (SEQ ID NO:32), or an allelic or species variation thereof.

7. A recombinant DNA molecule comprising a vector and the DNA sequence according to claim 2 or 3.

8. A cell that contains the recombinant DNA molecule according to claim 7.

9. A method of producing a polypeptide having an amino acid sequence corresponding to the human beta subunit of FcεRI, said method comprising culturing the cell according to claim 8 under conditions such that said

DNA molecule is expressed, and said polypeptide thereby produced.

10. The method of claim 9 further comprising isolating said polypeptide from the cell.

11. A method of expressing a complete and functional human $Fc_{\epsilon}RI$ receptor as characterized in mast cells comprising introducing into a host cell nucleic acid segments capable of encoding the human α, β, and γ subunits of $Fc_{\epsilon}RI$ and effecting expression of said segments under conditions such that said receptor is formed.

12. The method of claim 11 wherein the nucleic acid segments are DNA segments.

13. A nucleic acid segment capable of hybridization with a segment of the mRNA from which the human beta subunit is translated and, when so hybridized, capable of inhibiting said translation.

14. An antibody capable of binding to the beta subunit polypeptide of claim 5 or the amino acid sequence of claim 4 and neutralizing the $Fc_{\epsilon}RI$-related functions of the subunit.

15. The antibody of claim 14 which is a monoclonal antibody.

16. A nucleic acid segment comprising a hybridizing segment of at least approximately 14 nucleotides which is capable of hybridizing to a nucleic acid segment capable of encoding for at least an amino acid sequence comprising the aggregating functions of a human beta subunit under stringent conditions.

17. An amino acid sequence capable of binding to the amino acid sequence of the human beta subunit and inhibiting the aggregating functions of the subunit.

18. The nucleic acid segment of claim 16 further defined as capable of hybridizing to a mRNA capable of transcribing at least a fragment of the ß subunit.

19. A recombinant expression vector which comprises a nucleic acid sequence encoding the human ß subunit and from which said subunit is capable of being expressed when the vector is in a host cell.

20. A recombinant expression vector comprising a nucleic acid sequence selected from one of the following claims: 1, 13, 16, and 29.

21. A cell transfected with the nucleic acid segments of claims 1, 13, 16, or 29.

22. The transfected cell of claim 21 wherein the cell prior to transfection was selected from the group comprising CHO, COS, KU812, P815, Jurkett, 2M2 and 2B4 cells.

23. A method for determining the ability of a candidate substance to inhibit the formation or function of the human $Fc_\epsilon RI$, comprising:

(a) conferring on a host cell the ability to express the α, ß, and γ subunits of the human $Fc_\epsilon RI$;

(b) combining the host cell after step (a) with the candidate inhibitor substance;

(c) placing the combination in conditions suitable for expression of $Fc_\epsilon RI$;

(d) performing on the host cell candidate inhibitor combination of step c an assay for cell expression or for cell activation of a type which requires a functional human $Fc_{\epsilon}RI$; and

(e) determining whether the candidate substance has inhibited cell activation or receptor expression.

24. The method of claim 23 wherein the host cell is selected from the group consisting of CHO cells, T cells, KU818 and P815 cells, and the cell activation assay comprises measuring the phosphorylation of the $Fc_{\epsilon}RI$ receptor or of PLC-γ(phospholipase c-γ) by P^{32} label uptake.

25. The method of claim 23 wherein the host cell is selected from the group consisting of T cells, KU812 cells and P815 cells, and the cell activation assay comprises measuring calcium uptake response.

26. The method of claim 23 wherein the host cell is selected from the group consisting of T cells, KU812 cells and P815 cells, and the cell activation assay is the phosphatydil inositol metabolism.

27. A composition for the treatment of allergic diseases comprising human beta subunit inhibitor in a pharmaceutically acceptable carrier.

28. A method of treatment of allergic diseases in a human suffering therefrom, such method comprising administering to said human an amount of human beta subunit inhibitor effective to indirectly inhibit the binding of IgE to $Fc_{\epsilon}RI$ and to inhibit the aggregation function of the receptor or the signal transducing function related to allergic response.

29. A nucleic acid sequence coding for the amino acid sequence of claim 17.

FIG. 1A

```
TACTAAGAGT CTCCAGCATC CTCCACCTGT CTACCACCGA GCATGGGCCT ATATTTGAAG        60

CCTTAGATCT CTCCAGCACA GTAAGCACCA GGAGTCCATG AAGAAG ATG GCT CCT          115
                                                   Met Ala Pro
                                                     1

GCC ATG GAA TCC CCT ACT CTA CTG TGT GTA GCC TTA CTG TTC TTC GCT          163
Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu Phe Phe Ala
      5                  10                  15

CCA GAT GGC GTG TTA GCA GTC CCT CAG AAA CCT AAG GTC TCC TTG AAC          211
Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val Ser Leu Asn
 20                  25                  30                  35

CCT CCA TGG AAT AGA ATA TTT AAA GGA GAG AAT GTG ACT CTT ACA TGT          259
Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr Leu Thr Cys
                 40                  45                  50

AAT GGG AAC AAT TTC TTT GAA GTC AGT TCC ACC AAA TGG TTC CAC AAT          307
Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp Phe His Asn
            55                  60                  65

GGC AGC CTT TCA GAA GAG ACA AAT TCA AGT TTG AAT ATT GTG AAT GCC          355
Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile Val Asn Ala
        70                  75                  80
```

FIG. 1B

```
AAA TTT GAA GAC AGT GGA GAA TAC AAA TGT CAG CAC CAA CAA GTT AAT          403
Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln Gln Val Asn
     85                  90                  95

GAG AGT GAA CCT GTG TAC CTG GAA GTC TTC AGT GAC TGG CTG CTC CTT          451
Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp Leu Leu Leu
100                 105                 110                 115

CAG GCC TCT GCT GAG GTG GTG ATG GAG GGC CAG CCC CTC TTC CTC AGG          499
Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu Phe Leu Arg
                120                 125                 130

TGC CAT GGT TGG AGG AAC TGG GAT GTG TAC AAG GTG ATC TAT TAT AAG          547
Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys
            135                 140                 145

GAT GGT GAA GCT CTC AAG TAC TGG TAT GAG AAC CAC AAC ATC TCC ATT          595
Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile
        150                 155                 160

ACA AAT GCC ACA GTT GAA GAC AGT GGA ACC TAC TAC TGT ACG GGC AAA          643
Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys
    165                 170                 175

GTG TGG CAG CTG GAC TAT GAG TCT GAG CCC CTC AAC ATT ACT GTA ATA          691
Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile Thr Val Ile
180                 185                 190                 195
```

FIG. IC

```
AAA GCT CCG CGT GAG AAG TAC TGG CTA CAA TTT TTT ATC CCA TTG TTG        739
Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu
                200                 205                 210

GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA TTA TTT ATC TCA ACT CAG        787
Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile Ser Thr Gln
            215                 220                 225

CAG CAG GTC ACA TTT CTC TTG AAG ATT AAG AGA ACC AGG AAA GGC TTC        835
Gln Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe
        230                 235                 240

AGA CTT CTG AAC CCA CAT CCT AAG CCA AAC CCC AAA AAC AAC TGATATAATT     887
Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn Asn
    245                 250                 255

ACTCAAGAAA TATTTGCAAC ATTAGTTTTT TTCCAGCATC AGCAATTGCT ACTCAATTGT     947

CAAACACAGC TTGCAATATA CATAGAAACG TCTGTGCTCA AGGATTTATA GAAATGCTTC     1007

ATTAAACTGA GTGAAACTGG TTAAGTGGCA TGTAATAGTA AGTGCTCAAT TAACATTGGT     1067

TGAATAAATG AGAGAATGAA TAGATTCATT TATTAGCATT GTAAAAGAGA TGTTCAATTT     1127

CAATAAAATA AATATAAAAC CATGTAAAAA AAAAAAAAAA AAAAAAA                   1174
```

FIG. 2

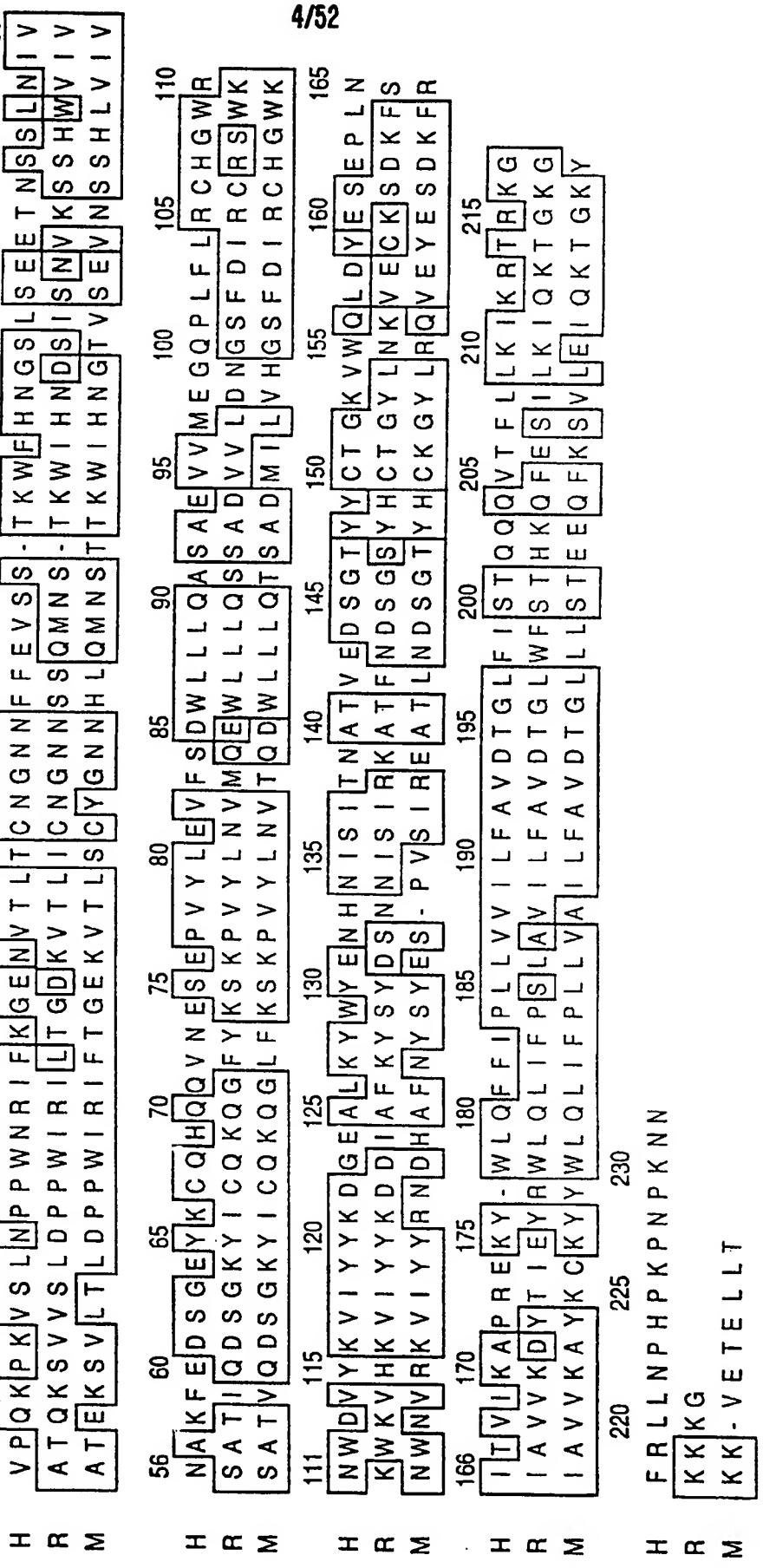


FIG. 3

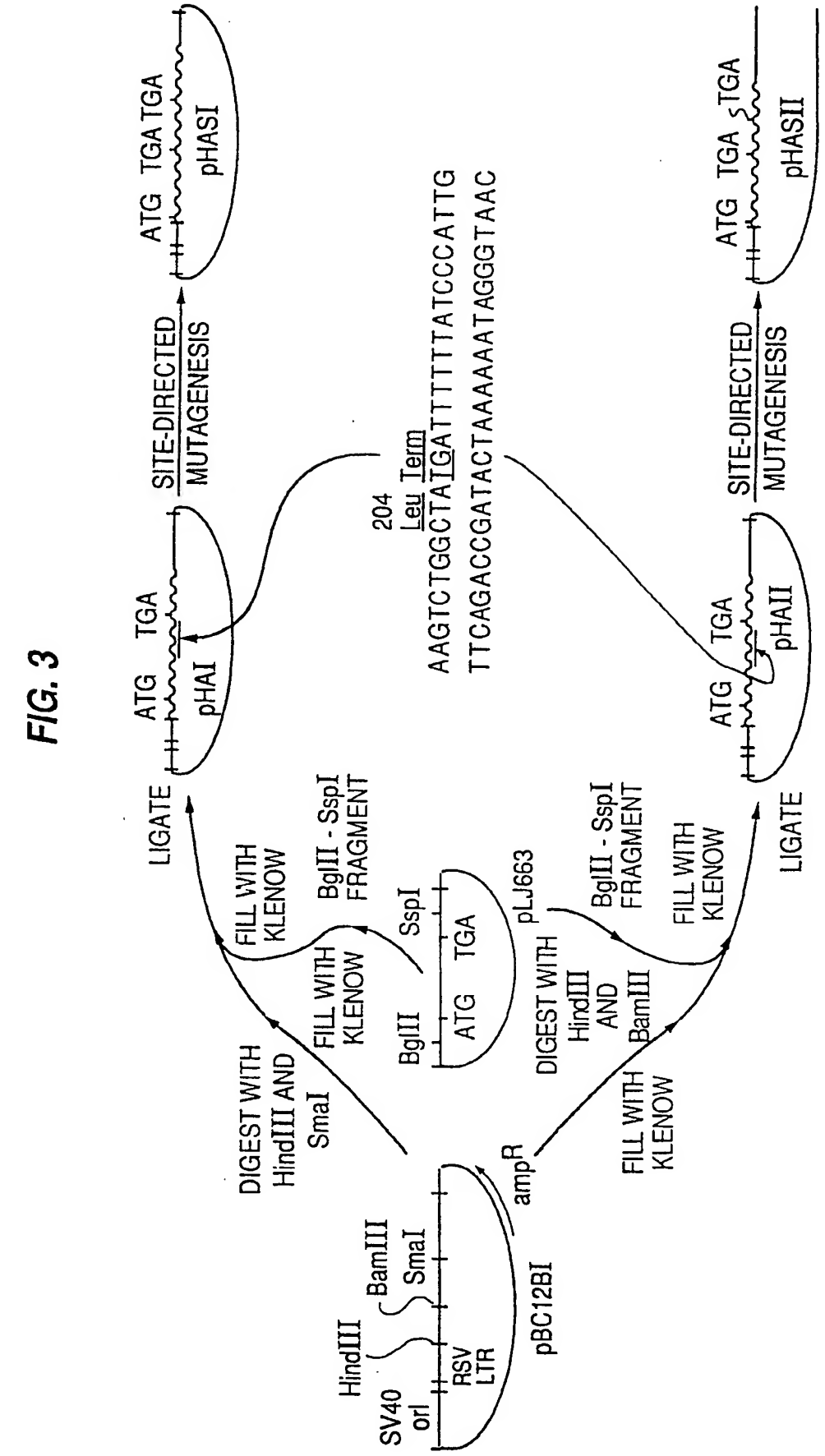
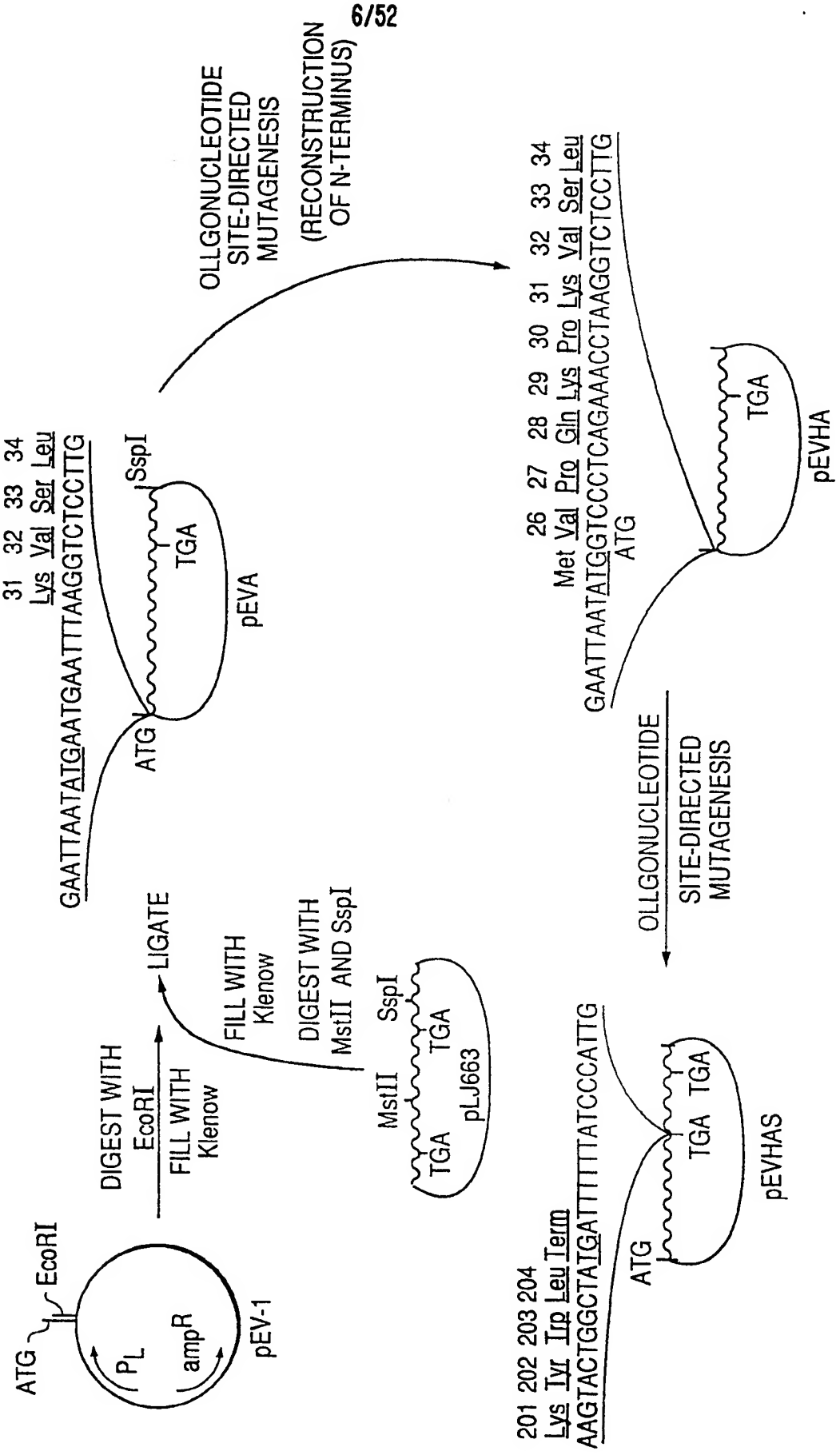

SV40 ori
RSV LTR
HindIII
BamIII
SmaI
pBC12BI
ampR
DIGEST WITH HindIII AND SmaI
FILL WITH KLENOW
BglII
SspI
BglII - SspI FRAGMENT
ATG TGA
pLJ663
DIGEST WITH HindIII AND BamIII
FILL WITH KLENOW
BglII - SspI FRAGMENT
FILL WITH KLENOW
LIGATE
ATG TGA
pHAI
SITE-DIRECTED MUTAGENESIS
ATG TGA TGA
pHASI
204
Leu Term
AAGTCTGGCTATGATTTTTTATCCCATTG
TTCAGACCGATACTAAAAAATAGGGTAAC
ATG TGA
pHAII
SITE-DIRECTED MUTAGENESIS
ATG TGA TGA
pHASII
LIGATE

FIG. 4


ATG
EcoRI
P_L
ampR
pEV-1
DIGEST WITH EcoRI
FILL WITH Klenow
LIGATE
FILL WITH Klenow
DIGEST WITH MstII AND SspI
MstII SspI
TGA TGA
pLJ663
31 32 33 34
Lys Val Ser Leu
GAATTAATATGAATGAATTTAAGGTCTCCTTG
ATG
SspI
TGA
pEVA
OLLGONUCLEOTIDE SITE-DIRECTED MUTAGENESIS
(RECONSTRUCTION OF N-TERMINUS)
26 27 28 29 30 31 32 33 34
Met Val Pro Gln Lys Pro Lys Val Ser Leu
GAATTAATATGGTCCCTCAGAAACCTAAGGTCTCCTTG
ATG
TGA
pEVHA
OLLGONUCLEOTIDE SITE-DIRECTED MUTAGENESIS
201 202 203 204
Lys Tyr Trp Leu Term
AAGTACTGGCTATGATTTTTTATCCCATTG
ATG
TGA TGA
pEVHAS

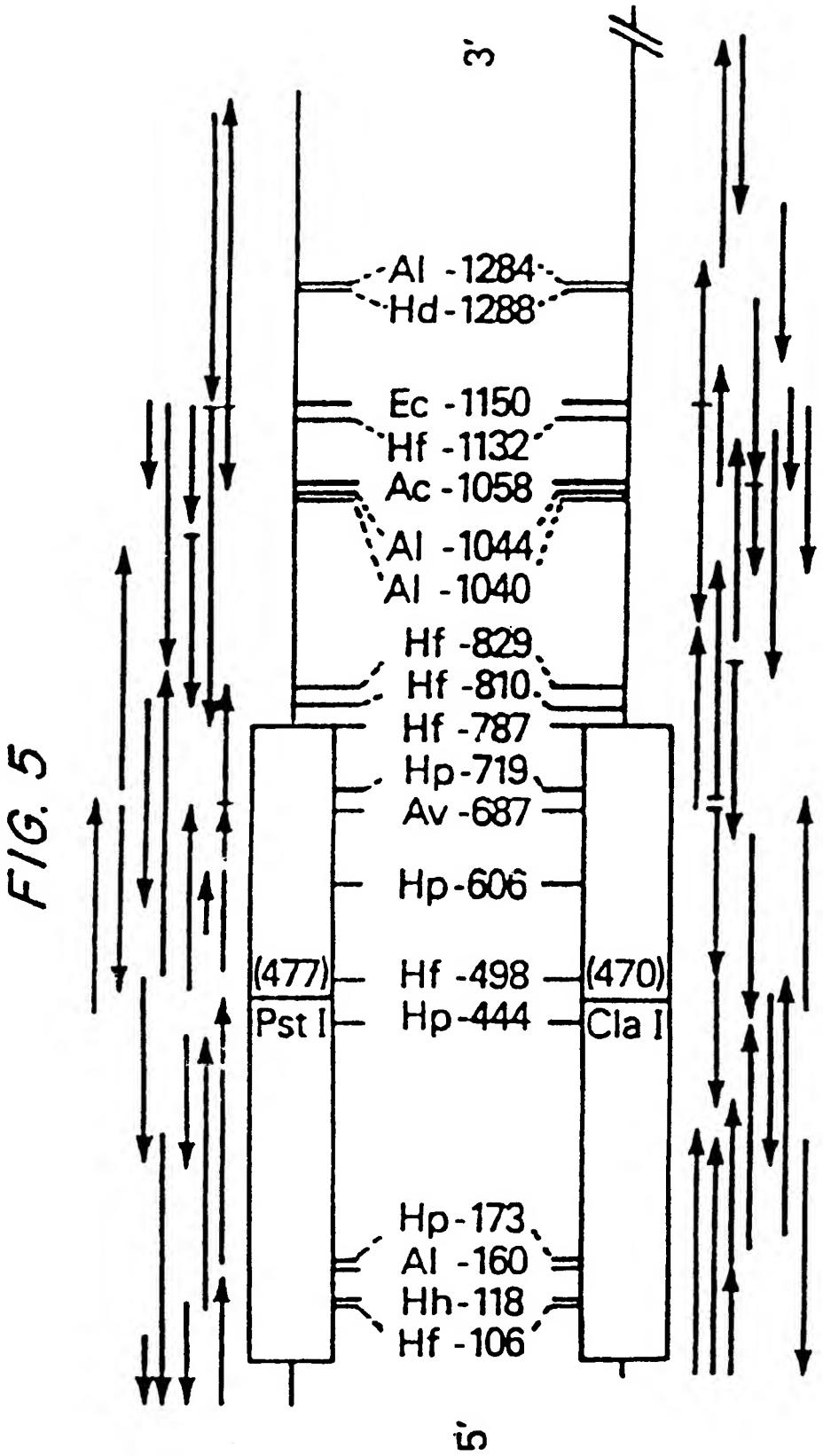

3'
Al -1284
Hd-1288
Ec -1150
Hf -1132
Ac -1058
Al -1044
Al -1040
Hf -829
Hf -810
Hf -787
Hp-719
Av -687
Hp-606
(477)
Hf -498
(470)
Pst I
Hp-444
Cla I
Hp-173
Al -160
Hh-118
Hf -106
5'

FIG. 5

FIG. 6A(1)

```
ACGTTTCTGT GTAACAATAT CTTTTATTCC TGGATAGTCC AATTA ATG AAA AAA          54
                                                      Met Lys Lys
                                                       -3

ATG GAC ACA GAA AAT AAG AGC AGA GCA GAT CTT GCT CTC CCA AAC CCA        102
Met Asp Thr Glu Asn Lys Ser Arg Ala Asp Leu Ala Leu Pro Asn Pro
  1               5                  10                  15

CAA GAA TCC CCC AGC GCA CCT GAC ATT GAA CTC TTG GAA GCG TCC CCT        150
Gln Glu Ser Pro Ser Ala Pro Asp Ile Glu Leu Leu Glu Ala Ser Pro
            20                  25                  30

CCT GCA AAA GCT CTA CCA GAG AAG CCA GCC TCA CCC CCA CCA CAG CAG        198
Pro Ala Lys Ala Leu Pro Glu Lys Pro Ala Ser Pro Pro Pro Gln Gln
        35                  40                  45
```

FIG. 6A(2)

```
ACA TGG CAG TCA TTT TTG AAG AAA GAG TTG GAG TTC CTG GGC GTA ACC        246
Thr Trp Gln Ser Phe Leu Lys Lys Glu Leu Glu Phe Leu Gly Val Thr
     50                  55                  60

CAA GTT CTG GTT GGT TTG ATA TGC CTT TGT TTT GGA ACA GTT GTC TGC        294
Gln Val Leu Val Gly Leu Ile Cys Leu Cys Phe Gly Thr Val Val Cys
 65                  70                  75                  80

TCC ACA CTC CAG ACT TCA GAC TTT GAC GAC GAA GTG CTT TTA TTA TAT        342
Ser Thr Leu Gln Thr Ser Asp Phe Asp Asp Glu Val Leu Leu Leu Tyr
                 85                  90                  95

AGA GCA GGC TAC CCA TTC TGG GGT GCA GTG CTG TTT GTT TTG TCT GGA        390
Arg Ala Gly Tyr Pro Phe Trp Gly Ala Val Leu Phe Val Leu Ser Gly
            100                 105                 110

TTT TTG TCA ATT ATG TCC GAA AGG AAA AAC ACA CTG TAT CTG GTG AGA        438
Phe Leu Ser Ile Met Ser Glu Arg Lys Asn Thr Leu Tyr Leu Val Arg
        115                 120                 125

GGC AGC CTG GGA GCA AAC ATT GTC AGC AGC ATC GCT GCA GGC TTG GGG        486
Gly Ser Leu Gly Ala Asn Ile Val Ser Ser Ile Ala Ala Gly Leu Gly
    130                 135                 140

ATC GCC ATA TTG ATT CTC AAT CTG AGC AAC AAC TCC GCT TAT ATG AAC        534
Ile Ala Ile Leu Ile Leu Asn Leu Ser Asn Asn Ser Ala Tyr Met Asn
145                 150                 155                 160
```

FIG. 6A(3)

```
TAC TGC AAG GAT ATA ACC GAA GAC GAT GGT TGC TTC GTG ACT TCT TTC          582
Tyr Cys Lys Asp Ile Thr Glu Asp Asp Gly Cys Phe Val Thr Ser Phe
                165                 170                 175

ATC ACA GAA CTG GTG TTG ATG TTG CTG TTT CTC ACC ATC CTG GCC TTT          630
Ile Thr Glu Leu Val Leu Met Leu Leu Phe Leu Thr Ile Leu Ala Phe
            180                 185                 190

TGC AGT GCC GTG CTG CTC ATT ATC TAT AGG ATT GGA CAA GAA TTT GAG          678
Cys Ser Ala Val Leu Leu Ile Ile Tyr Arg Ile Gly Gln Glu Phe Glu
        195                 200                 205

CGT AGT AAG GTC CCC GAT GAC CGT CTC TAT GAA GAA TTA CAT GTG TAT          726
Arg Ser Lys Val Pro Asp Asp Arg Leu Tyr Glu Glu Leu His Val Tyr
    210                 215                 220

TCA CCA ATT TAC AGT GCG TTG GAA GAC ACA AGG GAA GCG TCC GCA CCA          774
Ser Pro Ile Tyr Ser Ala Leu Glu Asp Thr Arg Glu Ala Ser Ala Pro
225                 230                 235                 240

GTG GTT TCA TAAGAATCAA GGGGCCAGGA CAATCTGATT CCAGTCTAGT                  823
Val Val Ser
```

FIG. 6A(4)

```
CTTGAGAGTC GATCTTTTTG CAACATTATG GCAACATTTC TGTTTCCTCC GCACTCTATC   883
AACTTTTCAA TTGGATTGTT CTGTAGATAC CCCTGTTTCA GTTATGATGC CTCTGGTCTT   943
TAATTATCTC CCTTTTTGTG GATATCGTTC AATCCAGTTT TCTTGTTTTG TGTCACAGTC  1003
TCACATACAA CCTTTCTGGA AAGTCATCAA AAACAAGCTA GCTTTTATTG CATGTCTACT  1063
TTCATGAACA AAAGGAAGGA GGAGTTATTT TGAGAGTTTA ACTAAACTTA GATAATCAGG  1123
TAATATTTGA CTCTTAGTTC ATTTTAGAAT TCTCAACAAT ACTTGTGCAT GATATATGCC  1183
CACCATATCA AGCCTTCTAT ATATATTTAA TATGGTATTT ACTTTTCTAT GTAGATAGAT  1243
TTTCCACCCT CAATAATAAT GGGTTTTTCA GAGACATAAA GCTTTATGAA AAGACACATA  1303
```

FIG. 6A(5)

```
TTATCTAATT CATGGGTATA TTCACTAATA CAGTTGTTGC TCAGTGGTGT TTACTACTTG    1363
GTGGGTAGTA GGTAATAGAG AACATTATTA AATCATTCAG TGTAGTGAGA TGCATAGGTA    1423
AAATCAGGGA CACTGTGAGT GTGTATATCT TTTGGTAAGA CATGTGTGAA AATGAAGAAT    1483
AAACTGATGA AGACTTGAGC TGGAAAGTAG TCAATGGGAA TGACAAGAAA TGATTGTGTA    1543
TAACACTTGT AGATAAATAA CTACCAACAA TTGGTAGAGA TTGCCATGTA TGCCTAAAAT    1603
CTCCCAGCCC AAGGCCAGCC TCTGTTACAC AGTGAGTTAG AGGCCAGTCT GGGCTACACA    1663
AGATCATACA TCAAAGGACG AAAGAAGATG TTGGTTCAAA CTGTTAACAC AGTAAGGGAT    1723
ATTTAAACAA ACAGAAGTTT GACTGATATA TTGAGTGCTT GAGTTTTTAA TAAAACTGAA    1783
TGAATAACAT TGCGGGGGAG GGGAGCAGTG ATGCAGAAGT CTGGATGATG GAGGAGTAGC    1843
AGAATCAGAT GAAACATTGA AACGTATTTC CAGACTTTTG TTCTGAGATG GTTATAAGAG    1903
```

FIG. 6A(6)

```
CAATCACCAT TAAATGAAGA AGGTCAAGAC ACCAAAAGAA TTATTTTGAG ATAGAATTAA    1963

GACAGTCAAA ATCCACATGC CTATACTTAG AAGGTGAAGT AAGGATCAAA AGTAGAAAGC    2023

CTAACGATTA GTTGGAAAAG CATATTACGT TAGGCAGCAG ATGTCTATAG TGGAGAAAAG    2083

TTAAACAAGG AGAAATAATG AACCACCAGA GACTCTACAT GTTGGTTTGG GAAATAAGAG    2143

AAAATAGCAA TTCTAAACGA ATGCAAACTC TGAAGAAGCA TTTCCCAAAG GGTGTGGGCA    2203

GAGGACCAGA ACATTTGCAA ATGTACCTAG AGAGCAAACC TGAATAGGAG GTAAAATGGG    2263

GGAAAAGCAG CTAAGAAAAT GATTTTGTTG CTGTTATTTA GATTTTAAAA GAAACAAAAA    2323

GAGTCATTAA AAATCTGTTT GCTGGGATCA GTTATTGTGT TCTCTGTGTA TGTCCAAAGT    2383

ACAGGTAACT TTTCTAAATC TTCCTGTAAG GCTCACCTCA TATGTCTCTT CACATAGCCA    2443

CACCCTTGAT TCACAGTTAC TCTACCACAG TAGTAAACTG TGCTTGTGGT CTCCCTTATG    2503

TATCTTCACT AGTGTTTATA AAATAAATCA GAATTATTTA AA                       2545
```

FIG. 6B

```
GTG AGA ACA TAT CTG TAATTG TTC TGAAATGATG CTAACCAGAG ATTTTATTTT        55
Val Arg Thr Tyr Leu
  1               5

AATCAAAGAC AACTAATTTT CTTTTAATCA AGTGCTTATC TCTAGCCTTT CAATAATATC     115

TACAGTTCTT CATTTATATG CACATAGCCA TCTATAAATG TAGTTTCCAA AGCACTCTCT     175

ACATATACTC ATTAACAAGA GCAAATACAC TCACCACAGT TAACTATGGT TTAACCCATT     235

ACTATACTTT TATTGACTGA AAACCTTGAG ACTGTACAAA AAAAAAAAAA A              286
```

FIG. 7D

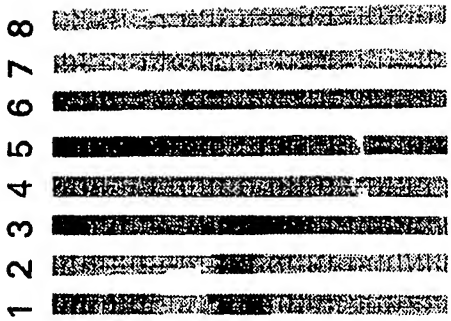


FIG. 7C

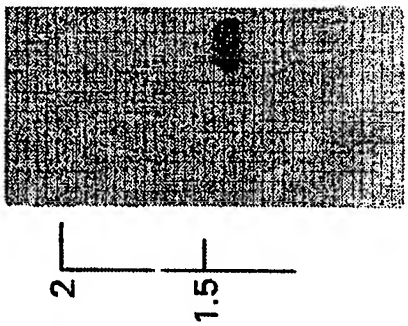


FIG. 7B

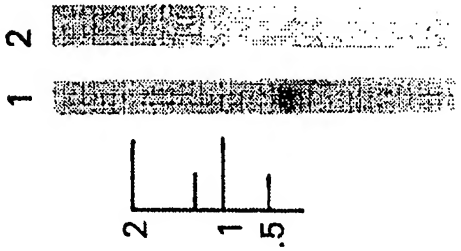


FIG. 7A

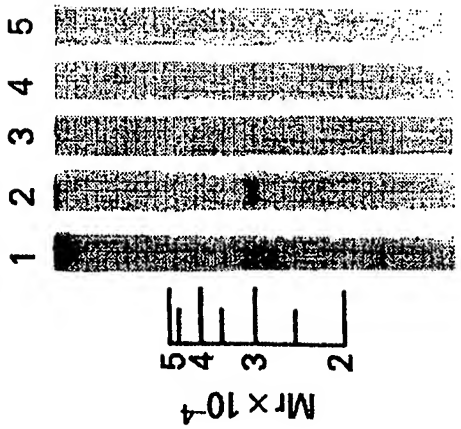


FIG. 8

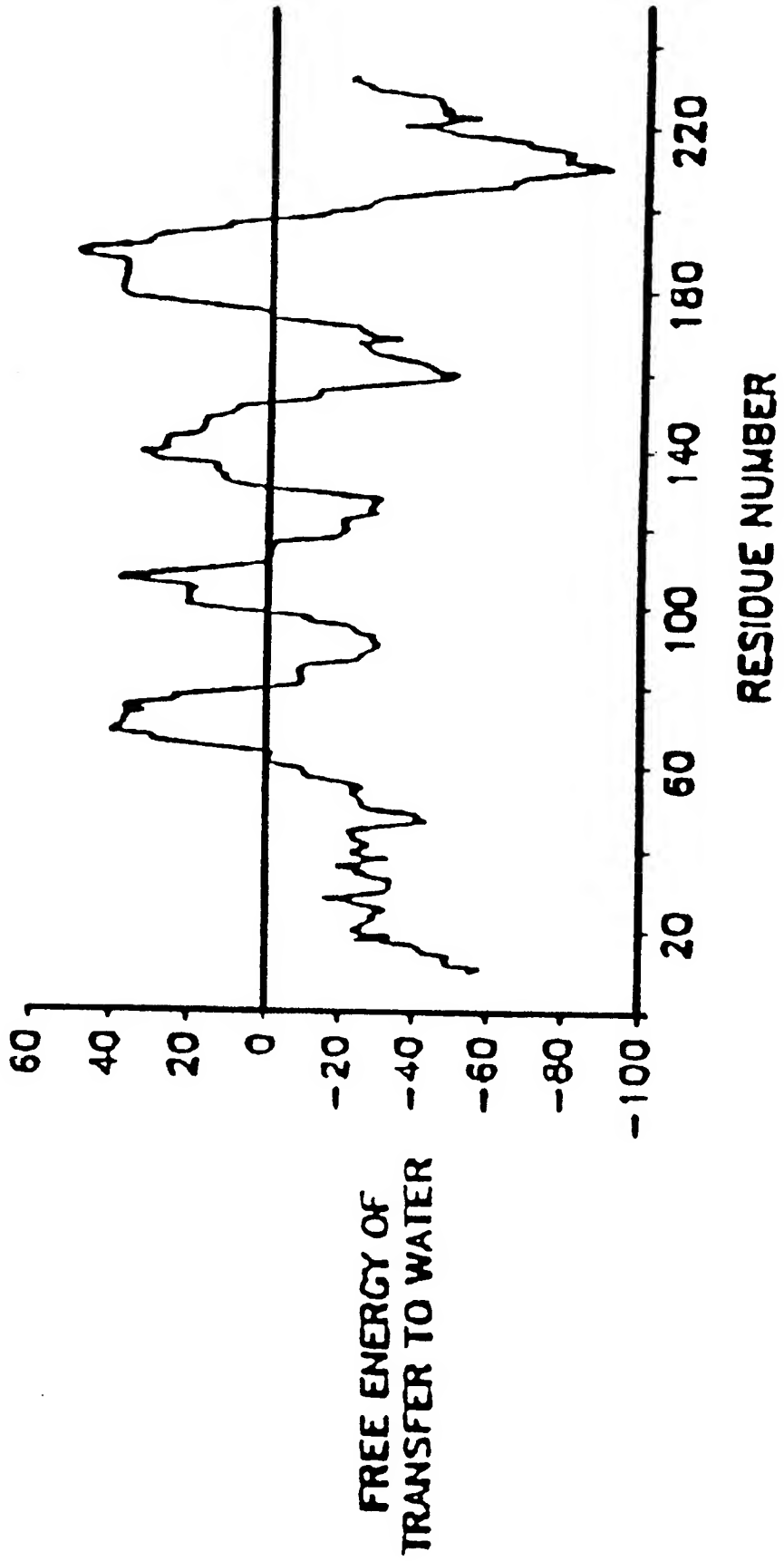

60
40
20
0
-20
-40
-60
-80
-100
20
60
100
140
180
220
RESIDUE NUMBER
FREE ENERGY OF TRANSFER TO WATER

FIG. 9

```
      AGCGCTGCAGCCCCCGCCCAGG ATG ATC CCA GCG GTG ATC TTG TTC      46
                             M   I   P   A   V   I   L   F      -11

TTG CTC CTT TTG GTG GAA GAA GCA GCT GCC CTA GGA GAG CCG CAG      91
 L   L   L   L   V   E   E   A   A   A | L   G   E   P   Q       5
                                      -1  +1

CTC TGC TAT ATC CTG GAT GCC ATC CTG TTT TTG TAT GGT ATT GTC     136
 L   C   Y   I   L   D   A   I   L   F   L   Y   G   I   V      20

CTT ACC CTG CTC TAC TGT CGA CTC AAG ATC CAG GTC CGA AAG GCA     181
 L   T   L   L   Y   C   R   L   K  {I   Q   V   R} {K   A      35

GAC ATA GCC AGC CGT GAG AAA TCA GAT GCT GTC TAC ACG GGC CTG     226
 D   I   A   S   R} {E   K   S   D   A   V   Y} T   G   L      50

AAC ACC CGG AAC CAG GAG ACA TAT GAG ACT CTG AAA CAT GAG AAA     271
 N   T   R  {N   Q   E   T   Y   E   T   L   K} | H   E   K      65

CCA CCC CAA TAG CTTTACAACACGTGTTCTCAGCTGCATTCCTTTTCCGCTTTTA     326
 P   P   Q   -                                                  68

ATTCTCTCCTCGCCCTCATGATTGACGTGGCTGTGCTACCTCCGTGCTTCTGGAACTAG     385
CTGACCTTATTCCCAGAACCATGCTAGGCTCTAAATCAATGTCCCCATATCCACCAAAG     444
ACTTACTCACTGACATTTCTCTTCTCCCATCCTCCTTTGCTTCATTCCTCTTTCCTTCC     503
CTGATCCTCTGTGCTCACTAAACAATGGGAAGGGATTACCCCCCAATAAAGCTGCCAGA     562
GATCACGCTCAAAAAAAAAAAAAA                                        586
```


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FIG. 10A

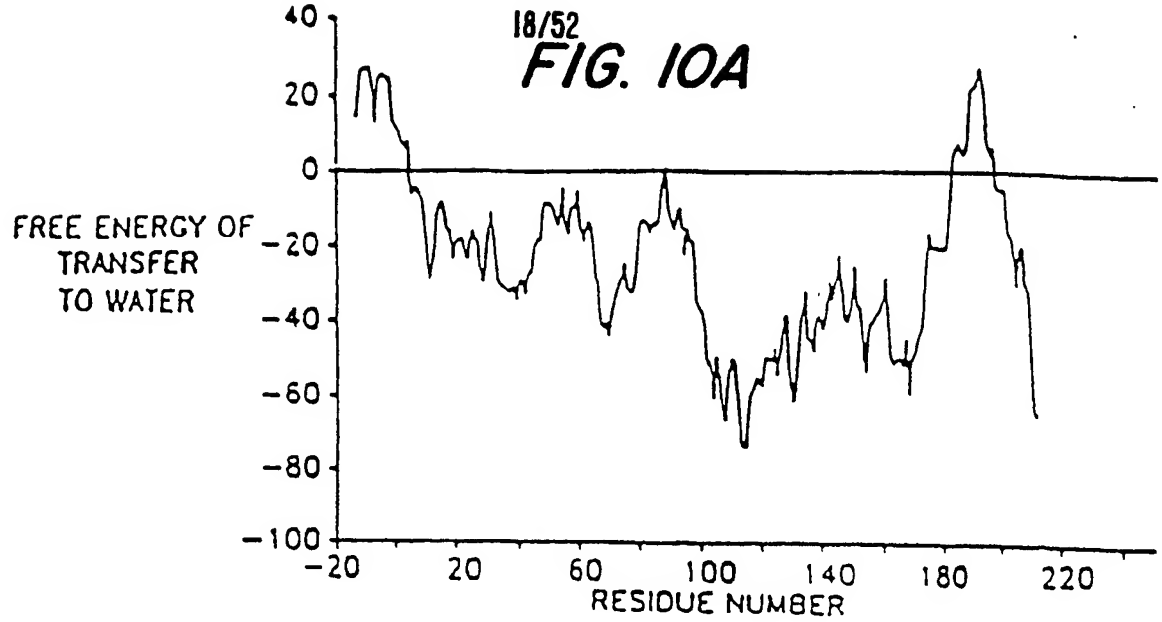


FIG. 10B

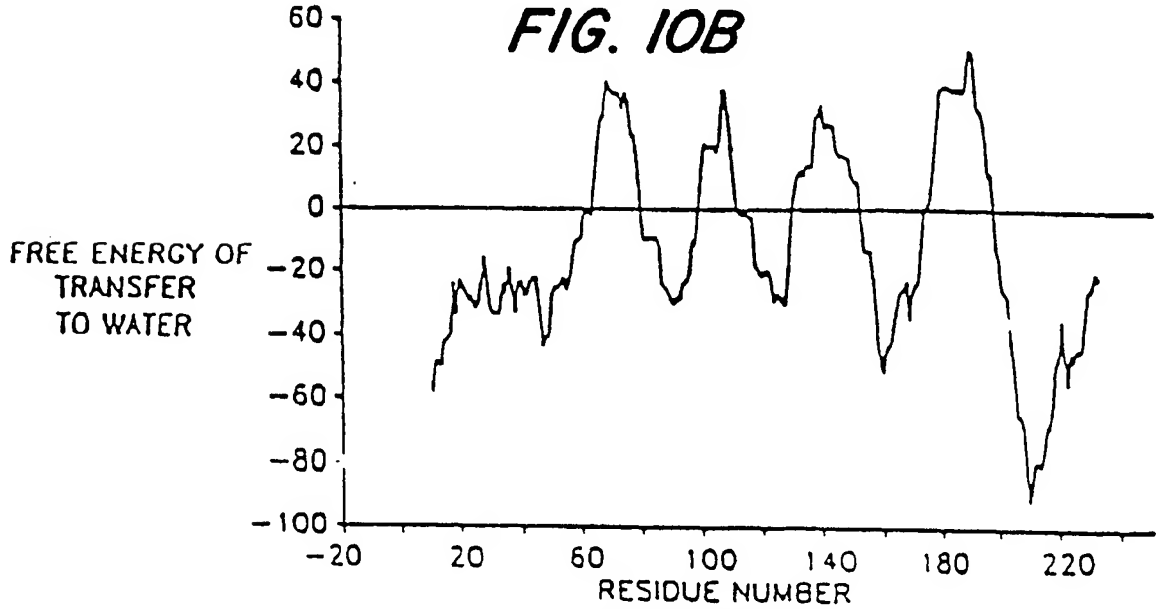
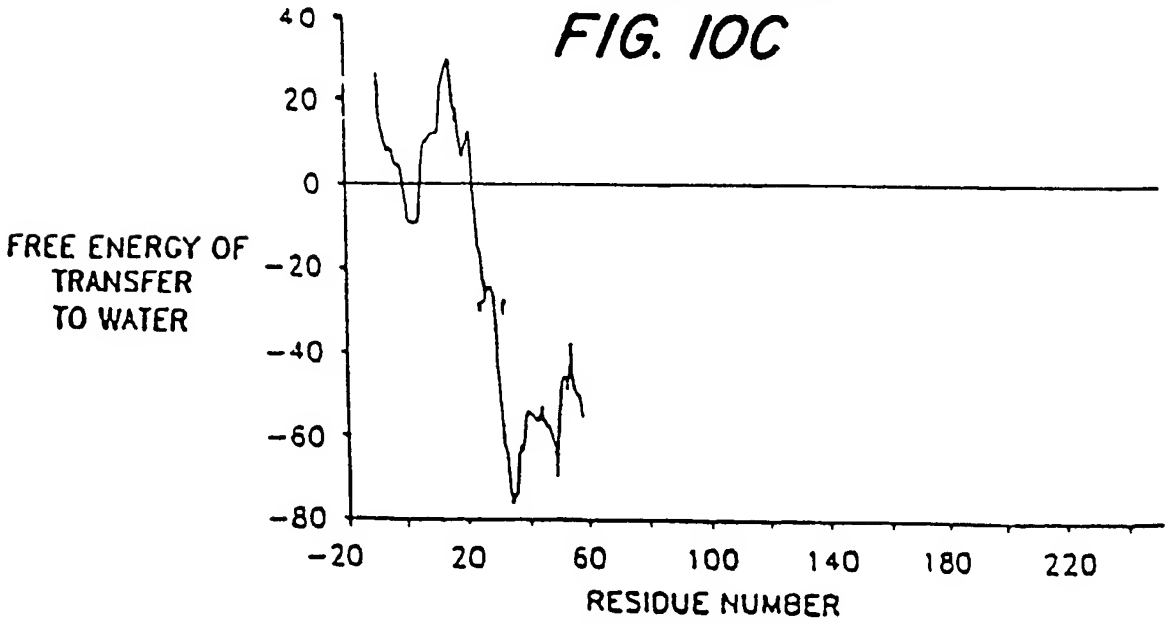


FIG. 10C



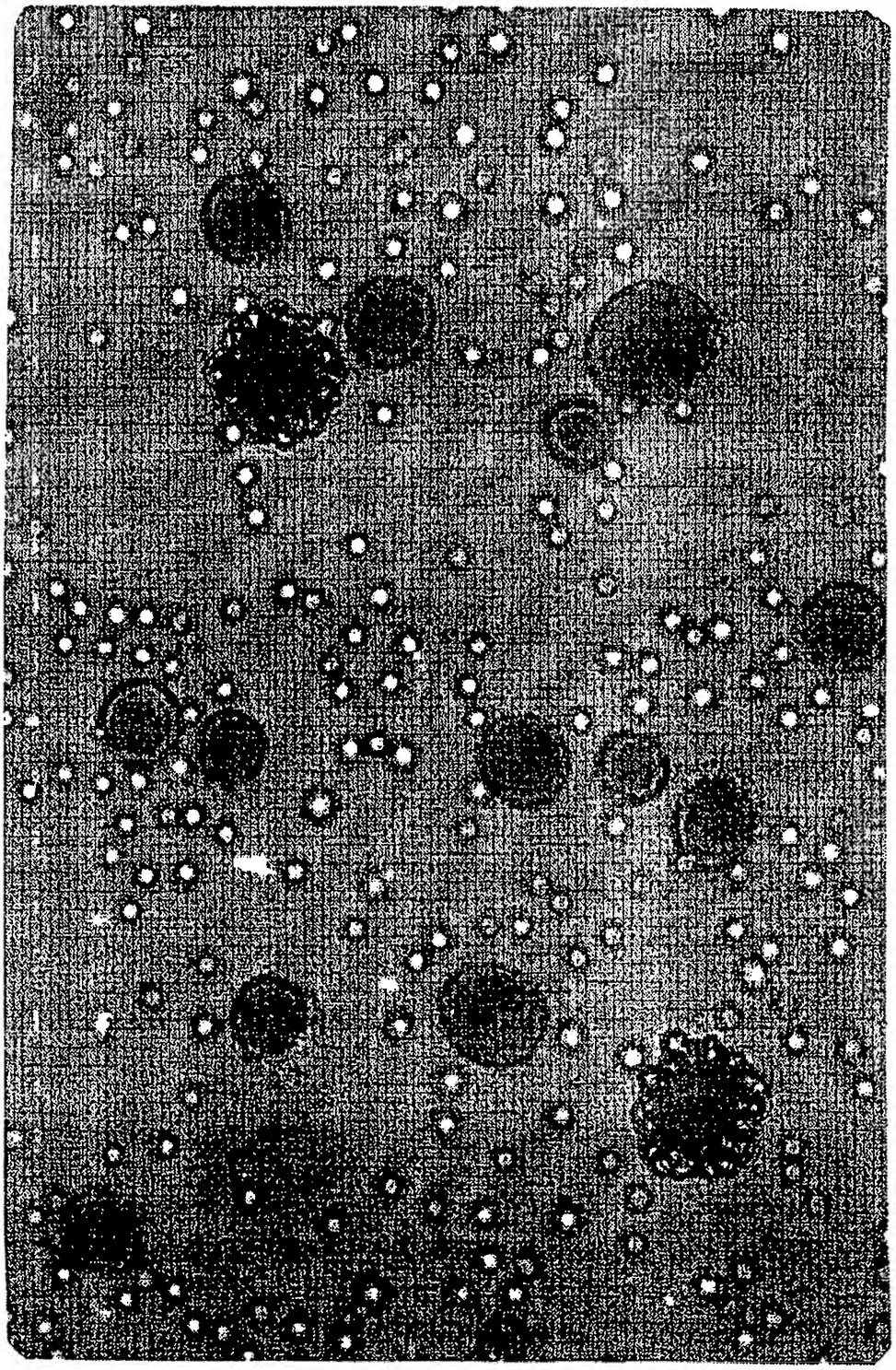


FIG.IIA

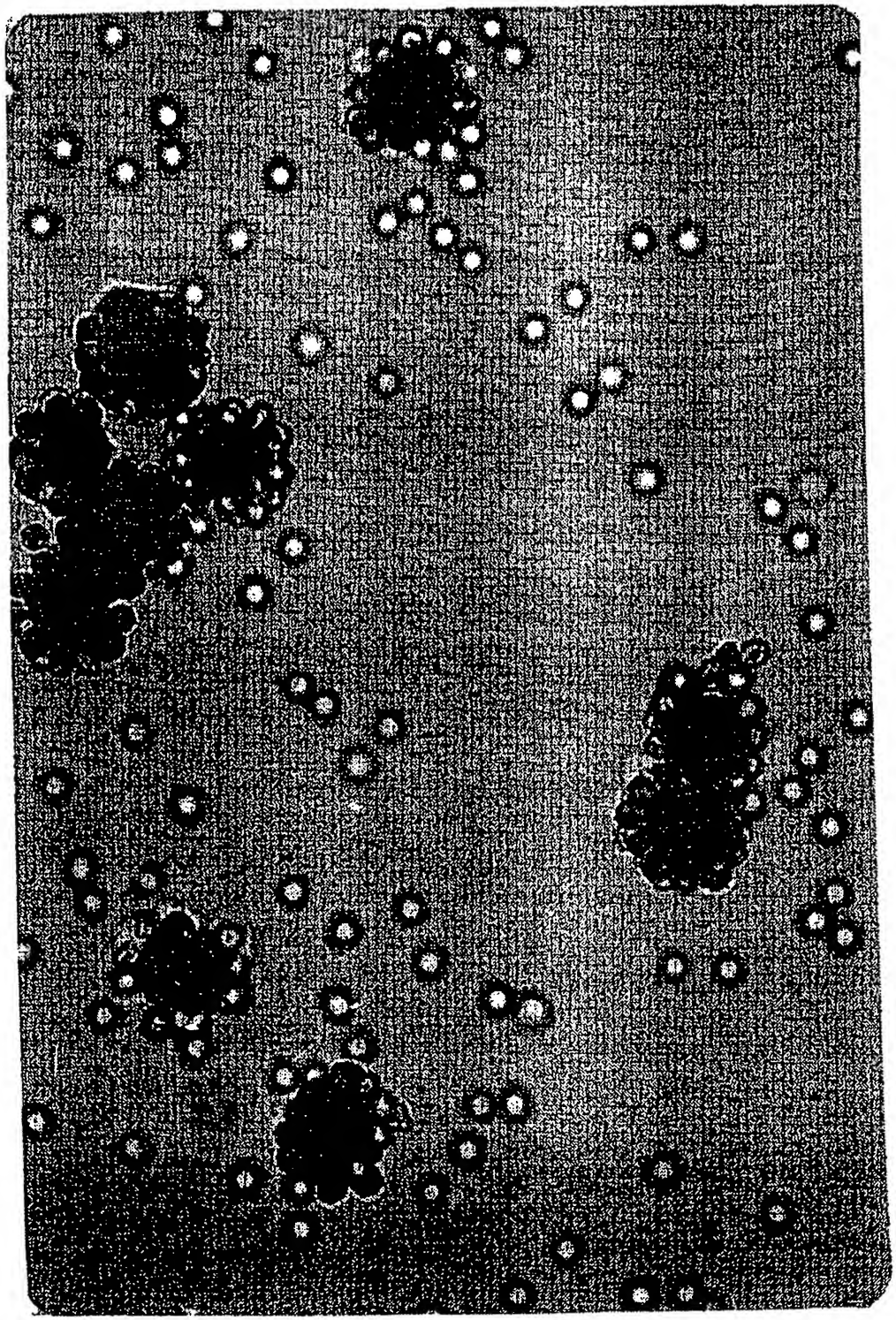


FIG.11B

FIG. 11C

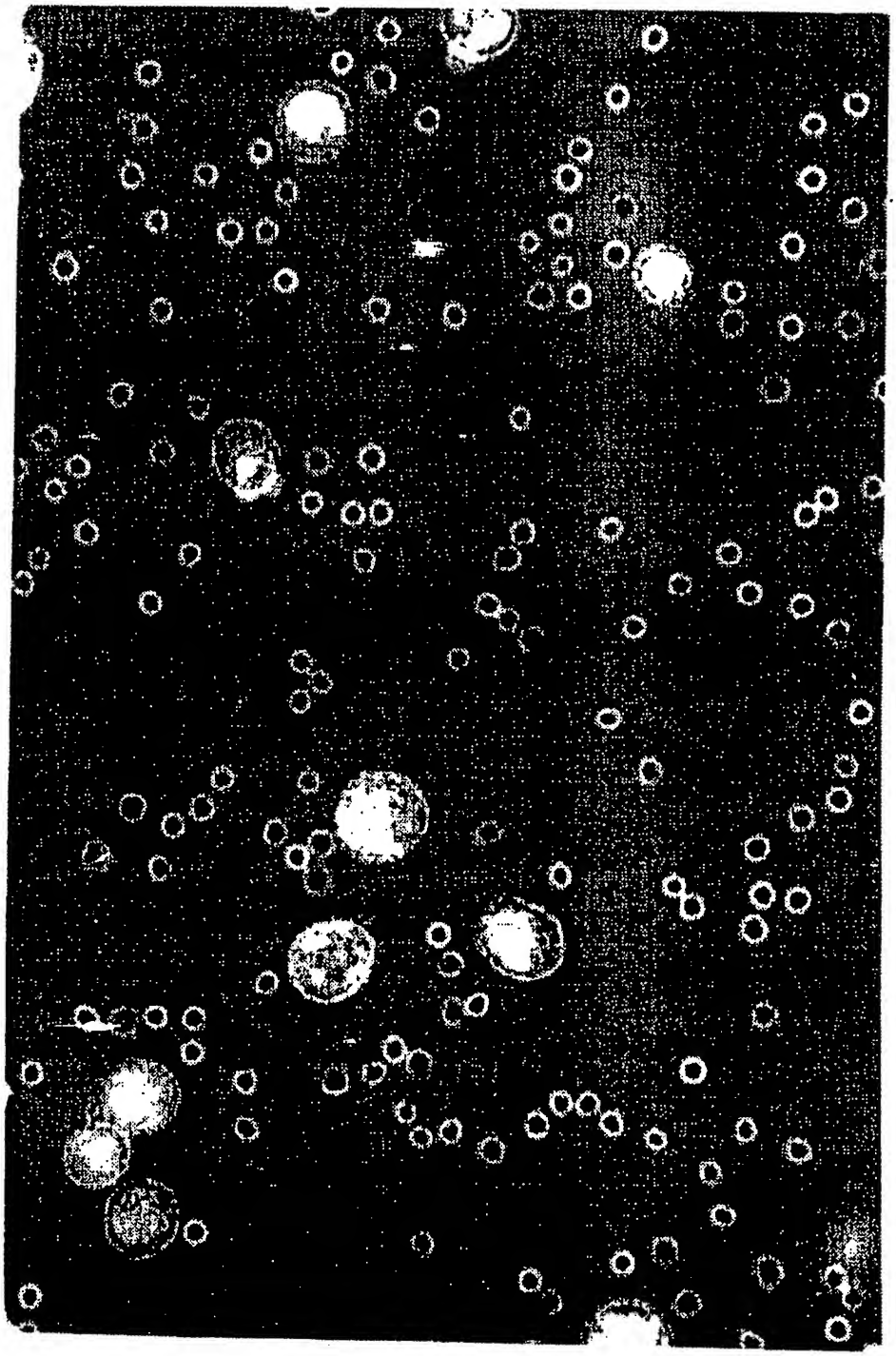
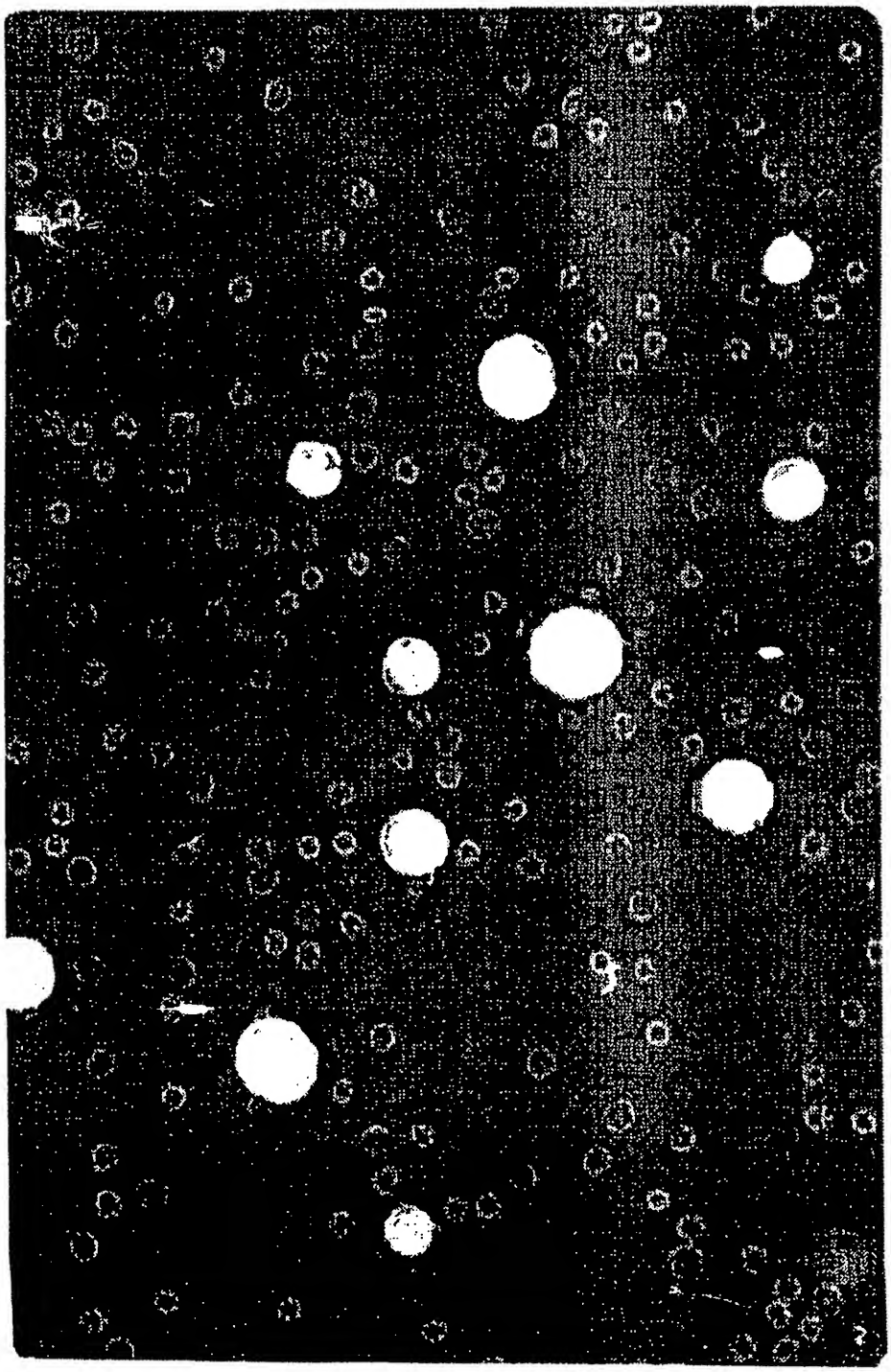


FIG. IID



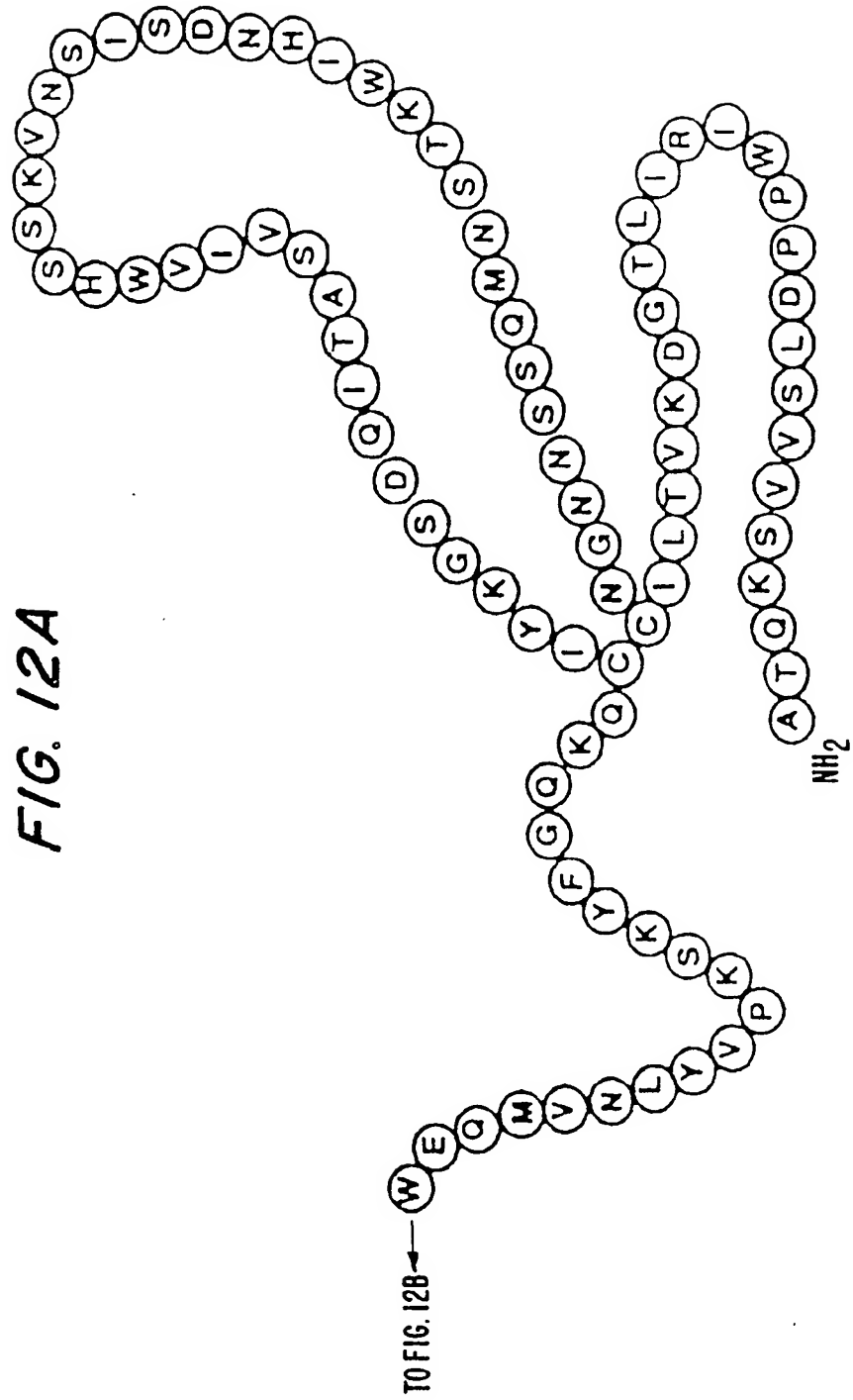


FIG. 12A

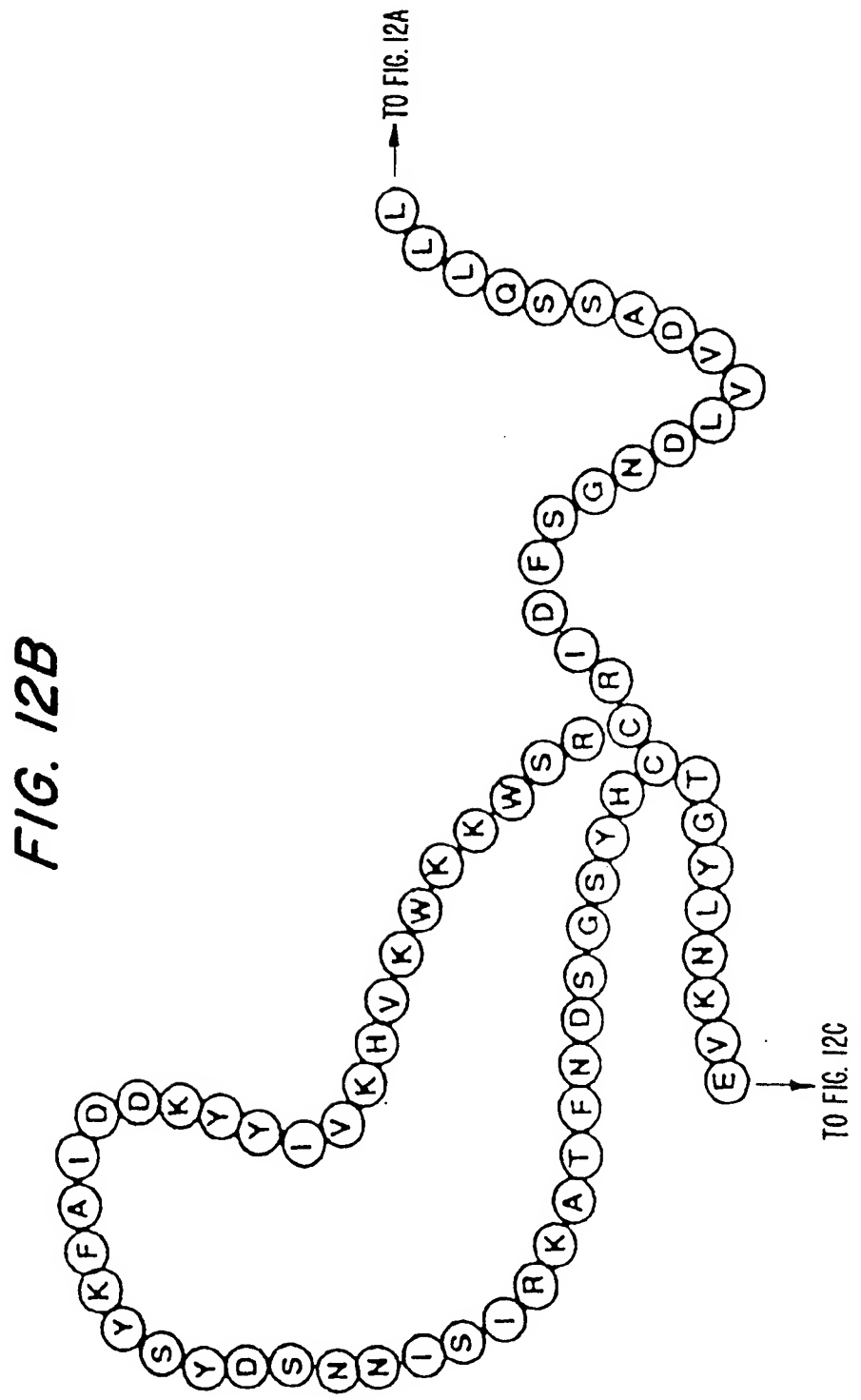

TO FIG. 12A
L L L Q S S A D V L V D F S G N D L V I D D K Y Y I V K H V K W K K W S R I C C H S Y G S D N F N A T K R I S I N N S D Y S Y K F A

FIG. 12C

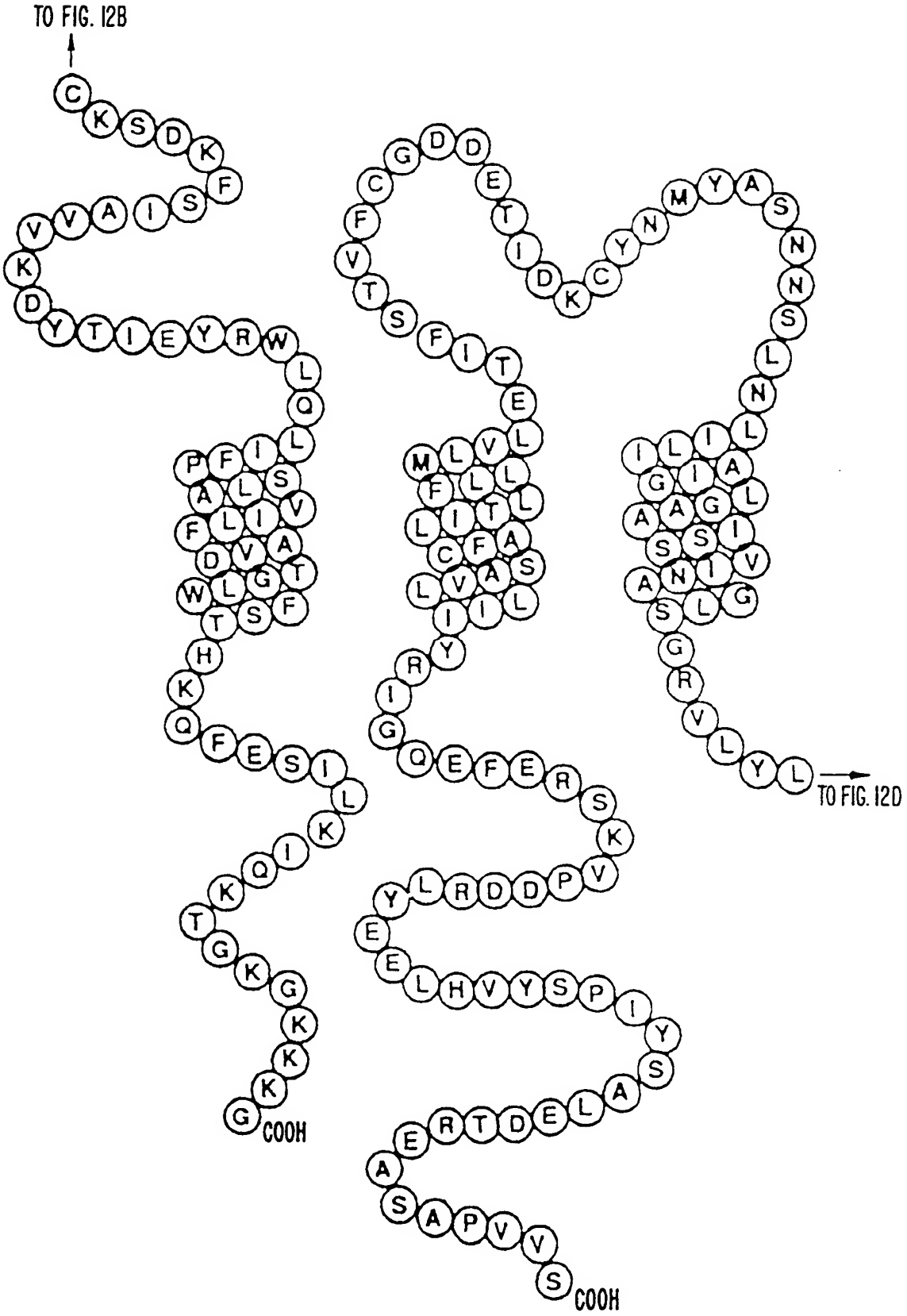


FIG. 12D

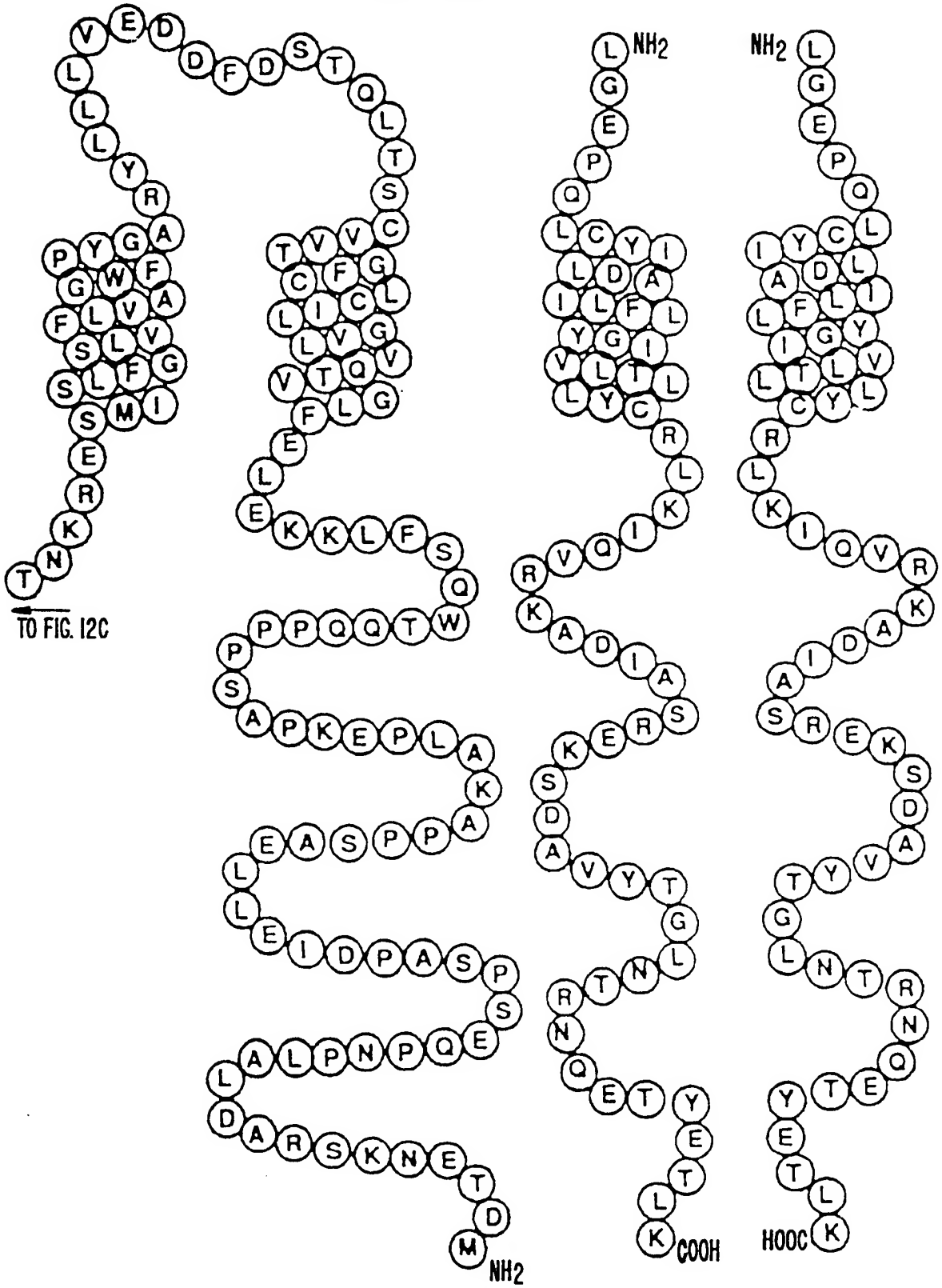


FIG. 13

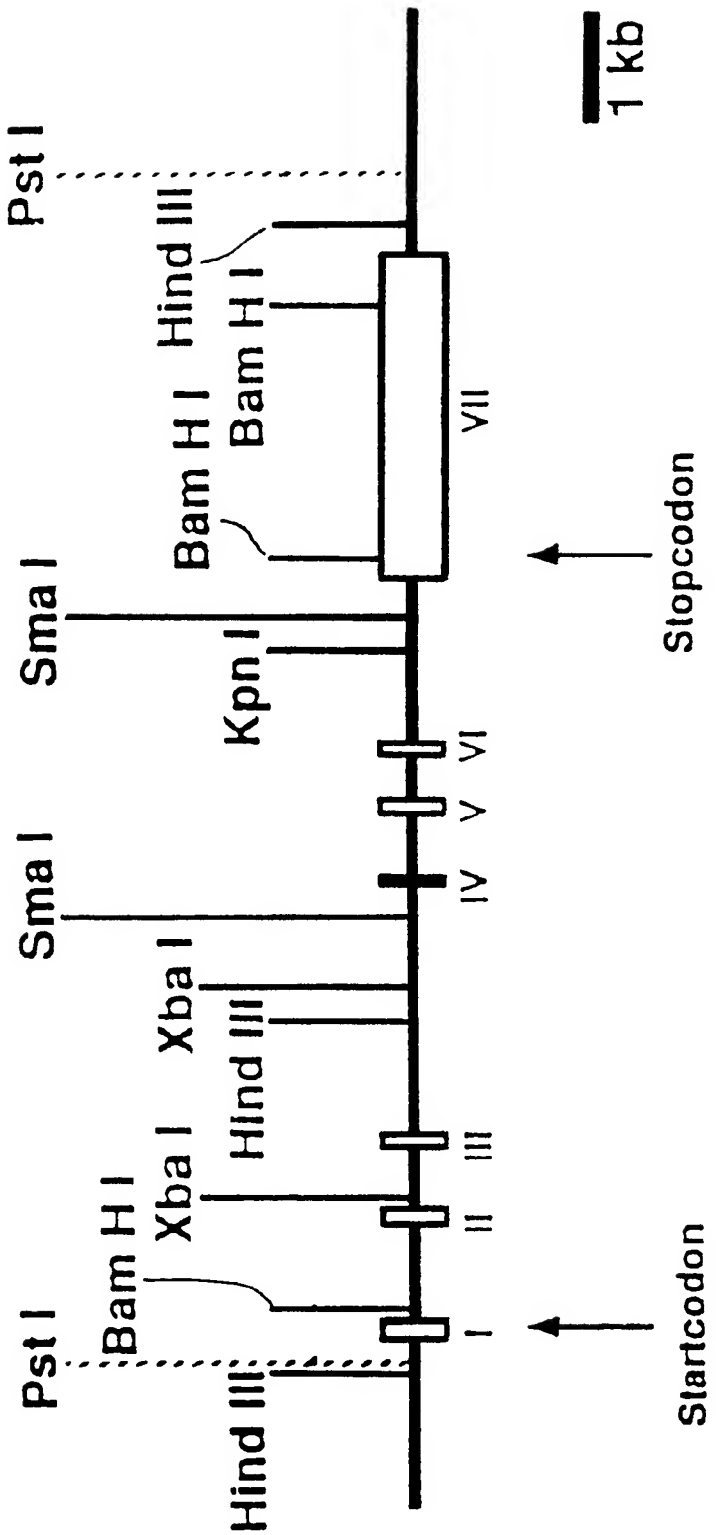

1 kb
Pst I
Hind III
Bam H I
Bam H I
Sma I
Kpn I
Stopcodon
VII
VI
V
IV
Sma I
Xba I
Hind III
III
Xba I
Bam H I
II
Pst I
Hind III
I
Startcodon

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FIG. 14A

```
AAGCTTTTCA AAGGTGCAAT TGGATAACTT CTGCCATGAG AAATGGCTGA ATTGGGACAC      60
AAGTGGGGAC AATTCCAGAA GAAGGGCACA TCTCTTTCTT TTCTGCAGTT CTTTCTCACC     120
TTCTCAACTC CTACTAAAAT GTCTCATTTT CAGGTTCTGT AAATCCTGCT AGTCTCAGGC     180
AAAATTATGC TCCAGGAGTC TCAAATTTTC TTATTTCATA TTAGTCTTTA TTTAGTAGAC     240
TTCTCAATTT TTCTATTCAT CACAAGTAAA AGCCTGTTGA TCTTAATCAG CCAAGAAACT     300
TATCTGTCTG GCAAATGACT TATGTATAAA GAGAATCATC AATGTCATGA GGTAACCCAT     360
TTCAACTGCC TATTCAGAGC ATGCAGTAAG AGGAAATCCA CCAAGTCTCA ATATAATAAT     420
ATTCTTTATT CCTGGACAGC TCGGTTAATG AAAAAATGGA CACAGAAAGT AATAGGAGAG     480
CAAATCTTGC TCTCCCACAG GAGCCTTCCA GGTAGGTACA AGGTATTATT TTTTTCTACC     540
```

FIG. 14B

```
CTCAGTCACT TGTGGCAGGG GAAGTCATAG TCACGGTGCT TAGGAGATGA AACTTTATTG     600
ATTTAGGCAT GGATCCATCT AGTTTAATTA ATATATTGGG TATGAGGAAG CTACTTGCTG     660
TACTTTCCAT GTGGTTCTCT CTCCCTGGAG AGGAACATTT TTACTCAGCT TGCAAACTGG     720
AAATAGATTT TCTCACATTA GAAGCTCATT TTCTGGGTAT GAGACAGGAG AGTTCATACT     780
GTGTATGTAG ATCTCTGGCT TCTGGGTCTG ACATGTGCTG AGGGACACAT ATCCTTCACA     840
CATGCTTTTA TAAATACTTG ATAAAGTAAC CTGCTTCTTG ATTGGTCTTT ATAATCCATA     900
AGCTGTGGGA TGCTTCTCTG AAGATGAAAA TAGTAATAGA GTCCCATCTA GCTATTCAAA     960
GCCATTCCTT CATTGTATTC TGTGCACATG AAGTTGGGGT TTGTTACTGA CAAAATATAT    1020
TCAGATACAT TTCTATGTTA AAAGGATTGT GAGATGCATA GGTAAATGTG TTTATTTTCA    1080
GTTTTACTTG TCAACATAGA TGAATGAGAA AGAACTTGAA AGTAACACTG GATTAAGAAT    1140
```

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FIG. 14C

```
AGGAAAATTT GGCATGGATT TTGCTCCATT TTGTCCCATC TAATCACTTG GATAGTGTTC    1200
AGGTGTTCTT GGTCAGTTAC TTGGATGCTC TGAGCTTTAG TTTCTTGGTG ATTACAATGA    1260
AGATTTGAAT TACAGGATGG CTTTGAAAAA ATAAACAAAA CTCCCCTTTC TGTCTGTCGA    1320
GAATGTTGCA CAGGGAGTTA CAGAATGTTC TCATGACTGA ATTGCTTTTA AATTTCACAG    1380
TGTGCCTGCA TTTGAAGTCT TGGAAATATC TCCCCAGGAA GTATCTTCAG GCAGACTATT    1440
GAAGTCGGCC TCATCCCCAC CACTGCATAC ATGGCTGACA GTTTTGAAAA AAGAGCAGGA    1500
GTTCCTGGGG GTGAGTGAGC CTCCTCCAAC TTTGACTAGA GTAAGGGTTG GGTCTAGAAA    1560
AGAATATTGA GTTGCATCAA CTGTTTTCCC ACTTGGATTC ATGAGAGGTG TTAGGTCCTT    1620
TAAAAAACAT GGTAGATAAA GAGTTGACAC TAACTGGGTC CTTTTGGGAA GAGCCAGAAG    1680
CATTTCCTCA TAAAGACTTT AAATTGCTAG GACGAGAATG GCCAACAGGA GTGAAGGATT    1740
CATAACTTTA TCTTTACTTA GATGTAAAGA ACAATTACTG ATGTTCAACA TGACTACATA    1800
CATAAAGGCG CATGGAGAAA AGTATTGGCC TTCCATGCAT TAGGTAGTGC TTGTATCAAT    1860
TCTTATAGTG GCTAGGGTAT CCTGGAAAAT CTTACGTGTG GATCATTTCT CAGGACAGTC    1920
TAGGACACTA ACGCAGTTTC TCATGTTTGG CTTCTATTAT TAAAAAATGA TACAATCTCG    1980
```

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FIG. 14D

```
GGAAAATTTT TTTGATTTTC ATGAAATTCA TGTGTTTTTC TATAGGTAAC ACAAATTCTG    2040
ACTGCTATGA TATGCCTTTG TTTTGGAACA GTTGTCTGCT CTGTACTTGA TATTTCACAC    2100
ATTGAGGGAG ACATTTTTTC ATCATTTAAA GCAGGTTATC CATTCTGGGG AGCCATATTT    2160
GTGAGTATAT ATCTATAATT GTTTCTGAAA TAACACTGAA CATAGGTTTT TCTCTTTCTC    2220
AGATCTAACC AGTTGTTTAT TCCCAGTATT AAGATGATAT TTATAATTCT TAATTATAAA    2280
TATATGTGAG CATATATAAC ATAGATATGC TCATTAACAA CAACAAAAGA TTCTTTTTAC    2340
AATTAACGGT GGGTTAAACA TTTAGCCCAC AGTTTTATCC CATGAGAAAC CTGAATCTAA    2400
TACAAGTTAA ATGACTTGCC TAAGGGCCAC TTGACTAATA GTAATTGAAC CTAAACTTTC    2460
AGAATCCAAC TCCAGGAACA TACTTCTAGC ACTATTCATC AATAAAGTTA TATGATAAAT    2520
ACATACAACT TTATCTGTCA ACTAAAAATA ACAACAGAGG CTGGGCATGG TGGCTCACAC    2580
CCGTAATCCC AGCACTTTGG GAGGCTGAGG CAGGTGGATC ACCTGAGGTC AGGAGTTTGA    2640
```

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FIG. 14E

```
GACCAGCCTG ACCAACATGG TGAAACCTCA TCTCTACTAA ATATAAAAAA TTAGCTGAGT    2700
GTGATAGTGC ATACCTGTAA TCCAGCTACT TAAGAGGCTG AGGCAGGAGG CTTGTTTGAA    2760
CCTGGAAGGC AGAGGTTGCA GTGAGCTGAG ATTGTGCCAT TGCACTCCAG CCTGGGCAAT    2820
AAGTGCGAAC TCTGTCTCAA AATAATAATA ATAATAATAG AAAATAAAGT TGTCTTCATG    2880
AAAAATGAGG AAAGAGATTG CTGGGGTGAG AAACATTAAG ATCAATGGGC ATATGGTGAC    2940
CTTCTATGCC CTAGAAACTC TTTTANGGTA TTTTCTCCTG GTATCTCTTT TACNCATCGT    3000
TCTATCTGGA AAAATAGGTG GATGAGTGAG ATAATAACGG TATATACTTT TTAAAGGTCT    3060
AATTGACATA TATAAATTGC AAGTATTTCA GATGTCAATT TGCTAACCTT GACACACATA    3120
GACACACATG AAAACATCAC CACATTAATA CAATGTATGT ATCCATCATT CCAAAAGCTT    3180
CCCTGTGTAT CTTTGTAACT CTTTCTTCCT CCCTCCACTC CTTGTCCTCT CGTTCCCAAG    3240
AAAACATTGA TCTGCTTCCT GTGAATATAA ATTAACTTAC ATTTTTTAGA GCTTTATATA    3300
AGTATGTTCT CTTTACTGTT TGTCTTCCTT CGCTGCACAG TTATTTTGAG ATTCTTCAAG    3360
```

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FIG. 14F

```
AGTATGTTCT CTTTACTGTT TGTCTTCCTT CGCTGCACAG TTATTTTGAG ATTCTTCAAG    3360
TTTTTTCTTT ATATCGATAC TTCATTCACA AGAATATATT TTAATTCTAG ACTATGTCAC    3420
ATTGACTTTG TCGTCTGCTA AATCCTTAGT GCTCAGATGA CTTGTTCAGG ACTCTCCTTG    3480
AACCTGTACC TCTGTTANAT TGAAACTTGT CTCTACTGTC TTTTTATTTC AAACACAGCT    3540
TATTAGGTGT CTCTCAACCC ATCAAACNCA CAATCTGAGT CTTTAGGAGA TTGCTTTGAA    3600
TTTGTGCTAT TGACTTATAT NTATATNAAA TNTGTAAATG TTTGGTAAAA ATATCATCAT    3660
GTACNTTTTC ATAATTACGC TATNTNCACA TGATATATGT CAGACTCTGG AAATATGCAT    3720
GCCACAGACA CGTGTTTCTT GCCTAAAGGG GCTGATGGAA GACNCACATA CNAATAGACG    3780
ATTGCAGTAG AATGAGAGTG GTGGTCTAAN CAGTACATGT CCTGATGTTG CTCGGACAGT    3840
TACTACNCCA AGAGTACCCC CTGCATTGTC AGGGTTAGCA TCTCCTGGAA GCCTCATGTA    3900
AATGAAGAAT TTCATGCTCC ATCCAGGACC TAATGAATAA GAATCTGCAT TTTAGCAAGA    3960
CCCTCATATG ATTCATATAC ACTTTTTTTT TTTTTTTTTA GATGGAGTCT CACTCTTGTC    4020
```

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FIG. 14G

```
GCCCAGGCTG GAGTGCAATG GCATGATCTT GGCTCACTGC AACCTCTGCC TCCCGGGTTC    4080
AAGTGATTCT CCTGTCTCAG CCTCCCTAGT AGCTGGGACT ACAGGTGCAT GCCACAGTGG    4140
CTGGCTAATT TTTGTATTTT TAGTAGAGAC AGGGTTTCAC CATTTTGGTC AGGCTGGTCT    4200
TGAACTCATG ACCTCCGGTG ATTCCCCCGC CTCGGCTTCC CAAAGTGCTG GGATTACAGA    4260
CATGAGCCAC CACACCCGCC TTATTCGTAT ACNCATTTAA TTCTGAGAAG CACTCTATAG    4320
AAAATAAGAA TAAGAAAATA TTGGGCTCAC AGGTGACATT AATAAGTAAC TTTATCGAGT    4380
ACCCCAAATT TTACCTATGT TTGGAAGATG GGGTTAAAAG GACACATTGA AAACAAGAAC    4440
TCATTGTGGC TTTTTTTTCC TCCTTTTTGA ACAGTTTTCT ATTTCTGGAA TGTTGTCAAT    4500
TATATCTGAA AGGAGAAATG CAACATATCT GGTGAGTTGC CCGTTTCTGT CTTTGTCCAT    4560
CCTTGAAAAG ATAAGAAGAA CAGAGTTTTA AGAGTCTTAA GGGAAACACA TCTTTGTCTC    4620
CTATATTACT TGTGAATGTG GATATATGAT TTTCTTTCAA TCTATTTTGT GTCCTAAGGC    4680
```

FIG. 14H

TTTTTGCAAC	AGAAGTTGGA	TATATCATTA	GAAACATAAA	TTGTACCATT	TAACATACAT	4740
GAAGTTTATG	TTTACCTTGA	CGTTCTTCTA	AAAAGTGTCC	TACACCGGCA	TTGTCCTTGT	4800
AGGCATATTC	ACATGATCAA	ATAAAATAAT	TAGTTTTCAA	TTAAGGAGAA	TATTTGAGGA	4860
AAGACCGTAC	GTGTTCATGT	GGTTCCTGAA	GGCAGTCCAG	TGAGAAAGTA	ATATATGCTT	4920
CATTAAACAA	TGCGGACATT	TTCAGGGTTT	CCCTTTTTAA	CCAAAATTTG	GAAGCAATGT	4980
GGAATTTACT	GGATGCATCC	AGCCCTGAAA	TGAAGATAGG	TTTATTGAAT	GTGCCAGCAA	5040
GTGCAGGCCC	AGGTCTGAGT	GTTCTTCATT	ATTATCAGGT	GAGAGGAAGC	CTGGGAGCAA	5100
ACACTGCCAG	CAGCATAGCT	GGGGGAACGG	GAATTACCAT	CCTGATCATC	AACCTGAAGA	5160
AGAGCTTGGC	CTATATCCAC	ATCCACAGTT	GCCAGAAATT	TTTTGAGACC	AAGTGCTTTA	5220
TGGCTTCCTT	TTCCACTGTA	TGTATTTTTT	TTTGTGTGGG	AAGACTAAGA	TTCTGGGTCC	5280
TAATGTAAGT	AAGAAGCCCT	CTTCTCCTGT	TCCATGAACA	CCATCCTTTT	CTGTAACTTC	5340

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FIG. 141

```
TATTACACAG TATAGTGGTT CTGTAAGTTC ACACAGCCCA GGGAGATGCT GGCTGCCCAC   5400
TCCCCTCAAC CCAGGCAAAT TCCTCGGGGT TAAAGTTATC TACTGCAAGT GACGATCTCT   5460
GGGTTTTTCT GTGCCTGTGT TTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTATGTGTCA   5520
CTTTAAAAGG ACTGGTCAGA TGGTAGGGAG ATGAAAACAG GAGATGCTAT AAGAAAATAA   5580
ACTTTTGGGG CGAATACCAA TGTGACTCTT TTTGTTTGTC ATTTGTTGCT GTTCAATAGG   5640
AAATTGTAGT GATGATGCTG TTTCTCACCA TTCTGGGACT TGGTAGTGCT GTGTCACTCA   5700
CAATCTGTGG AGCTGGGGAA GAACTCAAAG GAAACAAGGT AGATAGAAGC CCGATATAAA   5760
ATCTTGAATG ACAGGTTAAC GAATTGGAGC TTTATTCCTT AAAATATGGC CTGGGTTTTC   5820
TGAAACATTT CTTCCAGAAA ATAGTTTCTC CAAGTTTTAT TACTTTGGTT TACAAATCTC   5880
ACATTTAAAT CACATTTTAT ACCATAAGTA GCACACATTT CATAATATTC CTCTGAATGA   5940
GGGTTGGGAT AATAGGACTG ATATGTTAGA AATGCCTTAA AGTGTGTGGA GCATGAGAGA   6000
TGGATGTACA GAAGGCTTGT GAGGAAACCA CCCAGGTATC TGGCCTTGTT TTCTGCCCCA   6060
```

FIG. 14J

```
GAACTAGCCG CCTATTCCTC TTTCTGTTTT ATTCCTTTGT TTCTTGACTT TTCCTTTCCA   6120
ACTTGCTCTA AAACCTCAGT TTTCTTTCCT TTCTGATTCA TGACTACCAA ATGTTTTCAC   6180
TTGCCTCACC CGTCCATTAC ACCTTTGATA AGAACCACCA GACCTTGTGC TCATGTACTT   6240
GCCCATGTCT GATGGAAGAA ACATACTCTC TCCATCTGTC CACTTTCCTG AGGCATTCAA   6300
GTCTAGCCAC CTTTTAAAAT CACTCTCCTC CAGGCTGGGC ACGGTGTCAC GCCTGTAATC   6360
TCAGCACTTT GTGAGGCTGA GGAGGGCGGA TCACTTGAAG TCAGGAGTTC AAAACCAGCC   6420
TGGCCAAATG GCAAAACCAA ATCTTCTTCA ATTATAACCA AATCTTAAAC CAAATCTCTA   6480
CTAAAAAATA CAACAAAACA AAACAACAAC AACAAAAACA GAAAAGGAAA CATTAGCCCA   6540
GCGTGGTGGC AGGTACCTGA GGTTCCAGAT ACTTGGGAGG CTGAAGCAGG AGAATCGCTT   6600
GAGCCCAAGA GATGGAGGTT GCAGTGAGCC GAGATCATGC CACTGCACCA CAGCCAGGGT   6660
GACAGAGCCA TACTTCCCAG CACATTGGGA GGCCAAAGCT GAAGAATAAT TTGAGGTGAG   6720
```

FIG. 14K

```
GATTTGGAGA CCAGCCTGGC CAACATGGTG AAACTCCGTC TGTACTAAAA ATATAAAACT    6780
TAGTGGGGCA TGGGGGCACA CACCTGTAAT TTCAGCTACT TAGGAGGCTG AGGCAGGAGA    6840
ATTGCTTGAA CCCGGGAGGC GGAAGTTGCA GTGAGCCAAG ATCGTGGCCA CTGCACTCCA    6900
GCCTGGGTGA CATAGTGAGA TTCTGTCTCA AAAAAAATAA AAGAAATTTA AAAAATCACT    6960
CTCTTCCAAA GATAGATAAA TAAGACAGCA GATATACTAA GGAATAACCT CACCAACTTG    7020
TCATTGACTG ACATGATTTC TTTTGGCCCA CTTGGCCAGC TAGTCTGGTT TGGTTTTCTG    7080
GAAATGAAAG AAATAATCAG AGTTTAATGA CAGAGAGCGT GAGACCCAGA AAGACAAAAG    7140
TAGATGAGGT AAGTCTCTTG AGCGAGACTT CTAGGGATGG GAAATTTGTG GTGATTGATA    7200
TGAAATGATT TTTCCCTTAT CAGGTTCCAG AGGATCGTGT TTATGAAGAA TTAAACATAT    7260
ATTCAGCTAC TTACAGTGAG TTGGAAGACC CAGGGGAAAT GTCTCCTCCC ATTGATTTAT    7320
AAGAATCACG TGTCCAGAAC ACTCTGATTC ACAGCCAAGG ATCCAGAAGG CCAAGGTTTT    7380
```

FIG. 14L

```
GTTAAGGGGC TACTGGAAAA ATTTCTATTC TCTCCACAGC CTGCTGGTTT TACATTAGAT    7440
TTATTCGCCT GATAAGAATA TTTTGTTTCT GCTGCTTCTG TCCACCTTAA TATGCTCCTT    7500
CTATTTGTAG ATATGATAGA CTCCTATTTT TCTTGTTTTA TATTATGACC ACACACATCT    7560
CTGCTGGAAA GTCAACATGT AGTAAGCAAG ATTTAACTGT TTGATTATAA CTGTGCAAAT    7620
ACAGAAAAAA AGAAGGCTGG CTGAAAGTTG AGTTAAACTT TGACAGTTTG ATAATATTTG    7680
GTTCTTAGGG TTTTTTTTTT TTTTAGCATT CTTAATAGTT ACAGTTGGGC ATGATTTGTA    7740
CCATCCACCC ATACCCACAC AGTCACAGTC ACACACACAT ATGTATTACT TACACTATAT    7800
ATAACTTCCT ATGCAAATAT TTTACCACCA GTCAATAATA CATTTTTGCC AAGACATGAA    7860
GTTTTATAAA GATCTGTATA ATTGCCTGAA TCACCAGCAC ATTCACTGAC ATGATATTAT    7920
TTGCAGATTG ACAAGTAGGA AGTGGGGAAC TTTTATTAAG TTACTCGTTG TCTGGGGAGG    7980
TAAATAGGTT AAAAACAGGG AAATTATAAG TGCAGAGATT AACATTTCAC AAATGTTTAG    8040
TGAAACATTT GTGAAAAAAG AAGACTAAAT TAAGACCTGA GCTGAAATAA AGTGACGTGG    8100
```

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FIG. 14M

```
AAATGGAAAT AATGGTTATA TCTAAAACAT GTAGAAAAAG AGTAACTGGT AGATTTTGTT   8160
AACAAATTAA AGAATAAAGT TAGACAAGCA ACTGGTTGAC TAATACATTA AGCGTTTGAG   8220
TCTAAGATGA AAGGAGAACA CTGGTTATGT TGATAGAATG ATAAAAAGGG TCGGGCGCGG   8280
AGGCTCACGC CTGTAATCCC AGCCCTTTGG GAGGCCGAGG TGGGCAGATC ACGAAGTCAG   8340
TAGTTTGAGA CCAGCCTGGC CAACATAGTG AAACCCCGTC TCTACTAAAA ATACAAAAAA   8400
AAAATTAGCT GGGTGTGGTG GCAGTCACCT GTAGTCCCAG CTACTTGGGA GGATGAGGCA   8460
GGAGAATCGC TTGAACCTGG GAGGCGGAGG TTGCAGTGAG CCGAGATCGC ACCAGTGCAC   8520
TCCAGCCTTG GTGACAATGG GAGACTCCAT CTCAAAAAAA AAAAAAAAAA AAAAAAGATA   8580
AAAAGTCAGA AATCTGAAAA GTGGAGGAAG AGTACAAATA GACCTAAATT AAGTCTCATT   8640
TTTTGGCTTT GATTTTGGGG AGACAAAGGG AAATGCAGCC ATAGAGGGCC TGATGACATC   8700
CAATACATGA GTTCTGGTAA AGATAAAATT TGATACACGG TTTGGTGTCA TTATAAGAGA   8760
```

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FIG. 14N

```
AATCATTATT AAATGAAGCA AGTTAACACT CTAAGAGAAT TATTTTGAGA TAGAAGTGAA   8820
GCTAAGCTAA ACTTCACATG CCTATAATTG GAGGGAAAAA CTAAGGATAA AATCTAGCCT   8880
AGAAGATACA ATAATTAGTC ATAAACATGC ATTGTGAAAC TGTAGAGAGC AGGTAGCCCA   8940
AAATAGAGAA AGATTAGATA AAGAGAAAAT AAGTATCCAT CAGAGACAGT ATCTCTAGGC   9000
TTGGGCAAGA GAAAAGTCCA CAGTGATAAG CAACTCCACC TAAGGCATGA ATATGCGGCA   9060
GAGAAAACAG CAATAGTGAA TGAATGCAAA AGGTGCTGAG CAAATTCCAC ACATGAGTAT   9120
TGTGCATGAG TAAATGAATA AAACATTTGC AAAGACCTTT AGAGAAAGAG AATGGGAGCA   9180
TATGTGCGAA ATAAGATAGT TGATTATGAA TAGAAGGTAG TGAAGAAAAG CAAGCTAAGA   9240
AAAAATTCTG TTTATAAAAG AAGGAAAAGA TAGTTTATGT TTTTAGCCTA AGTATAAGAG   9300
TCCTACAGAT GGACTGAAAA AAATCAGTCT GAGAGTATTA GTCACAATTA ATGAAATAAT   9360
TACATTTTAT GTATTGAGGA TGCCAAGATT AAAAGGTGAC AGGTAGATGT TAATTTCCCT   9420
```

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FIG. 140

```
AGATTGTGAA AGTGATCACG ACAATCACAC AACAAATAAT TAAGTGACTT GGTATGCTTT   9480
ATTTAATTGT AGGGCCTGAG GTTTTCCATT CTCATTTTTC TAAAATACAA TTTTGTTTCT   9540
CCAAATTTGA CAGCAGAATA AAAACCCTAC CCTTTCACTG TGTATCATGC TAAGCTGCAT   9600
CTCTACTCTT GATCATCTGT AGGTATTAAT CACATCACTT CCATGGCATG GATGTTCACA   9660
TACAGACTCT TAACCCTGGT TTACCAGGAC CTCTAGGAGT GGATCCAATC TATATCTTTA   9720
CAGTTGTATA GTATATGATA TCTCTTTTAT TTCACTCAAT TTATATTTTC ATCATTGACT   9780
ACATATTTCT TATACACAAC ACACAATTTA TGAATTTTTT CTCAAGATCA TTCTGAGAGT   9840
TGCCCCACCC TACCTGCCTT TTATAGTACG CCCACCTCAG GCAGACACAG AGCACAATGC   9900
TGGGGTTCTC TTCACACTAT CACTGCCCCA AATTGTCTTT CTAAATTTCA ACTTCAATGT   9960
CATCTTCTCC ATGAAGACCA CTGAATGAAC ACCTTTTCAT CCAGCCTTAA TTTCTTGCTC  10020
CATAACTACT CTATCCCACG ATGCAGTATT GTATCATTAA TTATTAGTGT GCTTGTGACC  10080
TCCTTATGTA TTCTCAATTA CCTGTATTTG TGCAATAAAT TGGAATAATG TAACTTGATT  10140
```

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FIG. 14P

```
TCTTATCTGT GTTTGTGTTG GCATGCAAGA TTTAGGTACT TATCAAGATA ATGGGGAATT   10200
AAGGCATCAA TAAAATGATG CCAAAGACCA AGAGCAGTTT CTGAAGTCCT CCTTTTCATC   10260
AGCTCTTTAT CAAACAGAAC ACTCTATAAA CAACCCATAG CCAGAAAACA GGATGTAGGA   10320
ACAATCACCA GCACACTCTA TAAACAACCC ATAGCCAGAA AACAGAATGT AAGGACAATC   10380
ACCAGCCATC TTTTGTCAAT AATTGATGGA ATAGAGTTGA AAGGAACTGG AGCATGAGTC   10440
ATATTTGACC AGTCAGTCCT CACTCTTATT TACTTGCTAT GTAAACTTGA GAAAGCTTTT   10500
TTCTCTTTGT GAACCTCAGG TTTTACATCT GAAAATGAGA AATTTGGAAC AAAAGATTCC   10560
TAACTGGTCT TTCTGTTCCC ATATTCTGTG ATTTTTCAAT ATTTAGGATT TTTGGTAATC   10620
ACAATTACTT AGTTTGTGGT TGAGATAGCA ACACGAATCA GAACTATTTG GTGGACATAT   10680
TTTCAAAGGA GTAGCTCTCC ACTTTGGGTA AAGAAGTGAT GCNGGTCGTG GTGGCTCACG   10740
```

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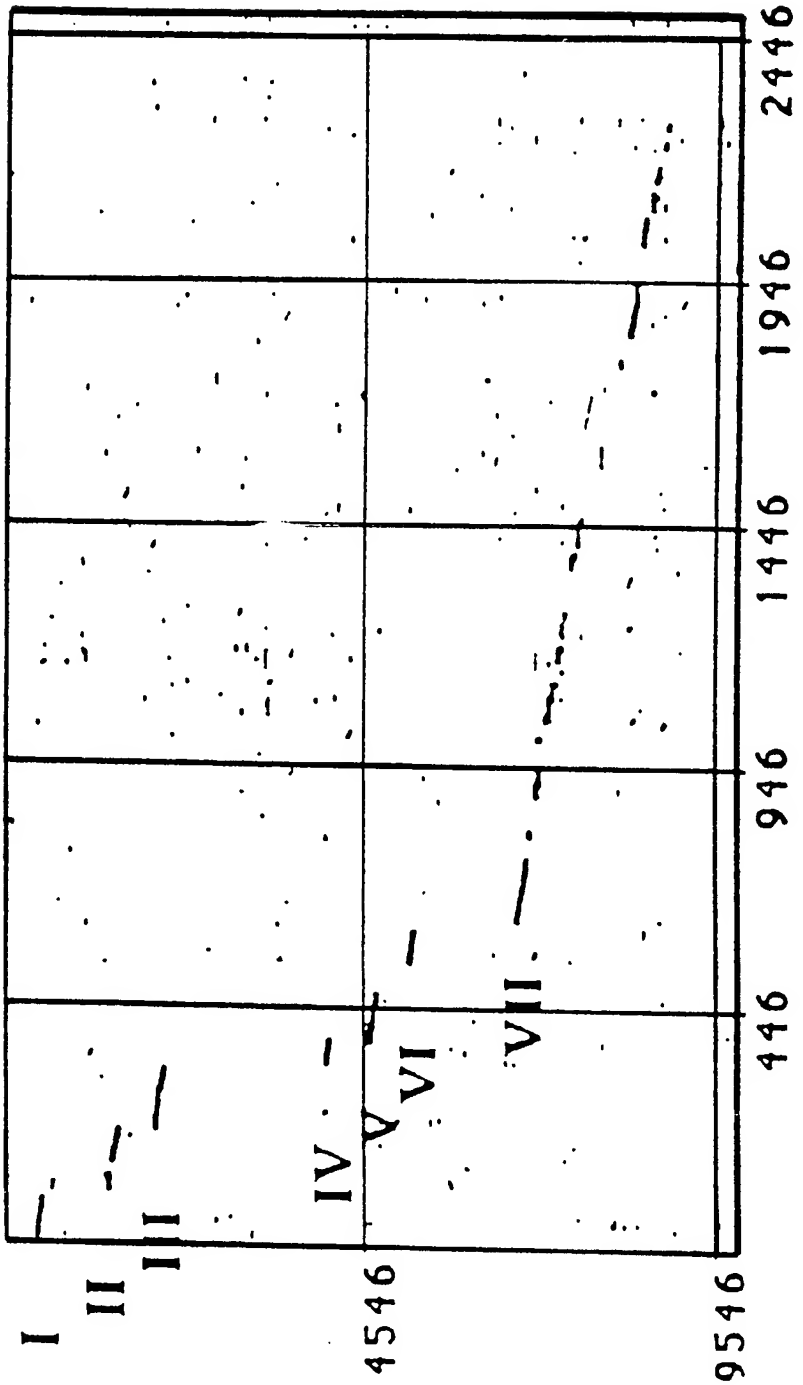
FIG. 14Q

```
CCTGTAATCC CAGCACTTTA GGGAGGCCAA GGCGGGTGGA TCACGAGGTC AGGAGATCGA   10800
GACCATCCTG GCTAACACGG TGAAACCCCG TCTCTACTAA AAAATACAAA AAATTAGCCA   10860
GGCGTGGTGG CGGGCGCCTG TAGTCCCACG TACTCGGGAG GCTGAGGCAG GAGAATGGCA   10920
TGAACCAGGG AGGCGGAGCT TGCCGTGAGC CGAGATAGCG CCACTGCAGT CCCTCCTGGG   10980
CAAAAGAGCA AGACTGCGTC TCAAAAAAAA AAAAAAAAAA AAAAAAAGAA GTGTGTGGAG   11040
TAGCAGGACA CCTGCAACAA TAATATTTTT CTAAATCCCT CTGAAAAATG CTAATCAAAG   11100
GGTTTTTTTC CTAAAAATTG TCTTAGAAAT AAAATTTCCC CTTTGGGAGA CCGAGGCTGG   11160
CAGATCACGA GGTCAGGAGA TAGAGACCAC GGTGAAACCC CGTCTCTACT AAAAATACTA   11220
AAAATTAGCC GGGGNGTGGT GGTGGGTACA CCTGTAGTCC CAGCTACTTG GAGGCTGAGG   11280
CTGGAGAATC ACGTGAAC                                                 11298
```

FIG. 15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1946
1446
946
446
β rat cDNA
I
II
III
IV
V
VI
VII
4546
9546
β human gene

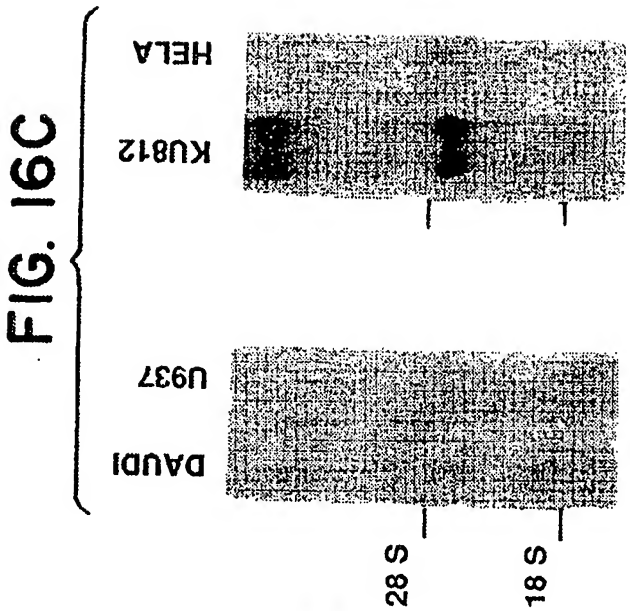


FIG. 16C

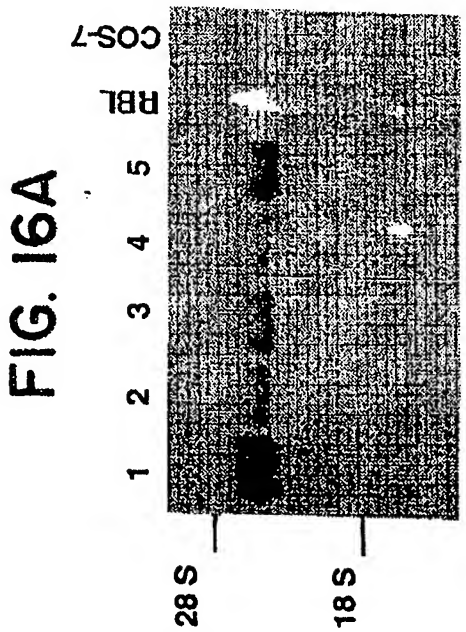


FIG. 16A

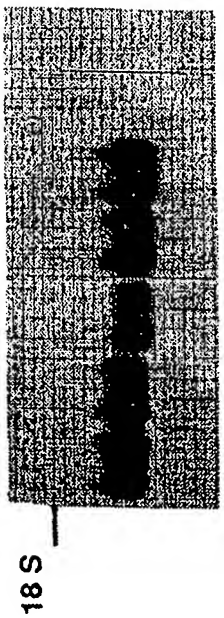


FIG. 16B

FIG. 17A

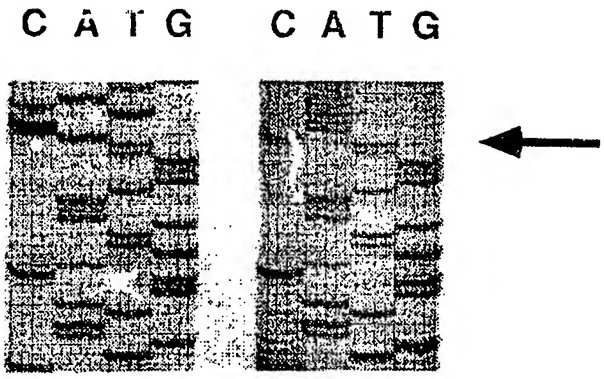


FIG. 17B

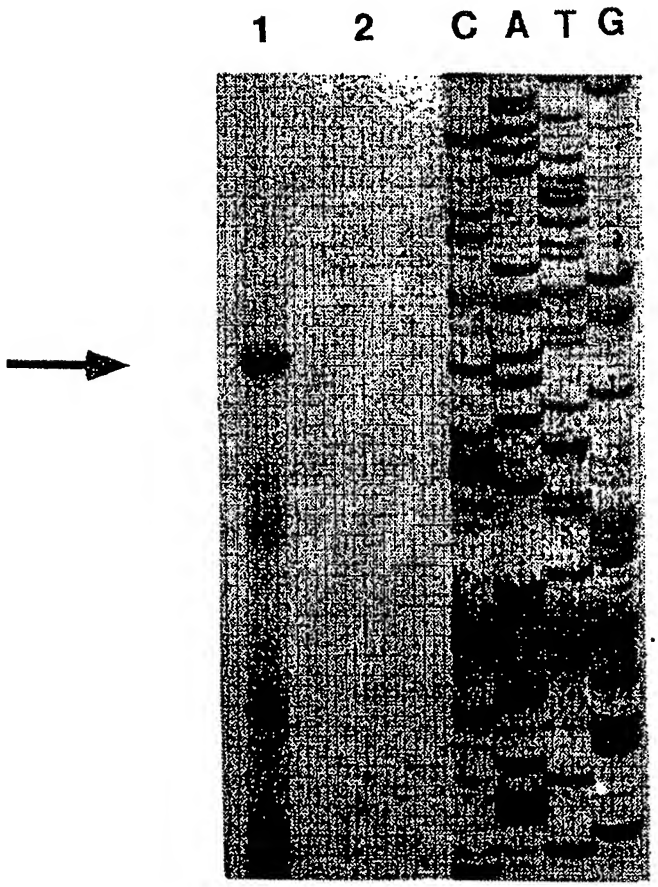


FIG. 18

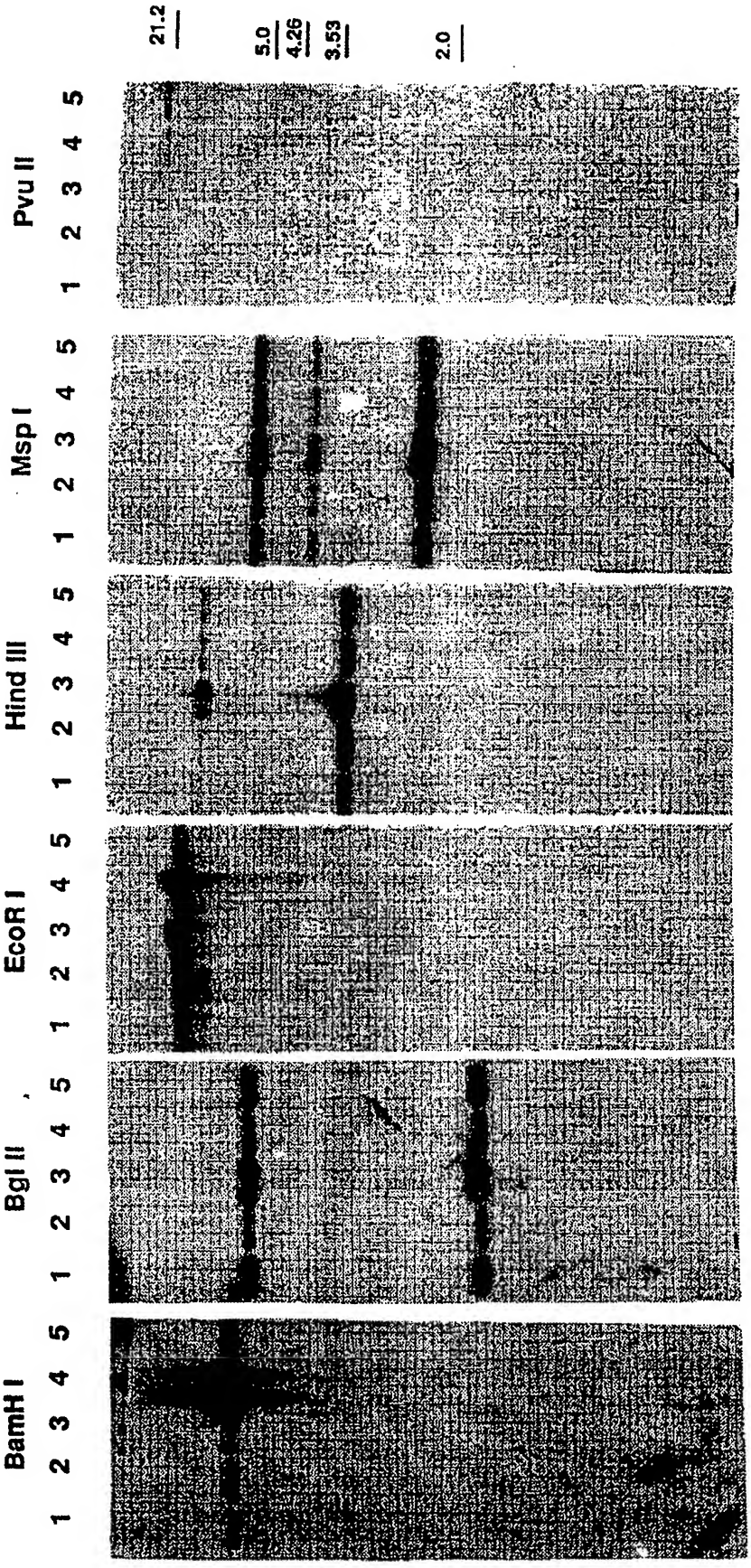

21.2
5.0
4.26
3.53
2.0
Pvu II
1 2 3 4 5
Msp I
1 2 3 4 5
Hind III
1 2 3 4 5
EcoR I
1 2 3 4 5
Bgl II
1 2 3 4 5
BamH I
1 2 3 4 5

FIG. 19A

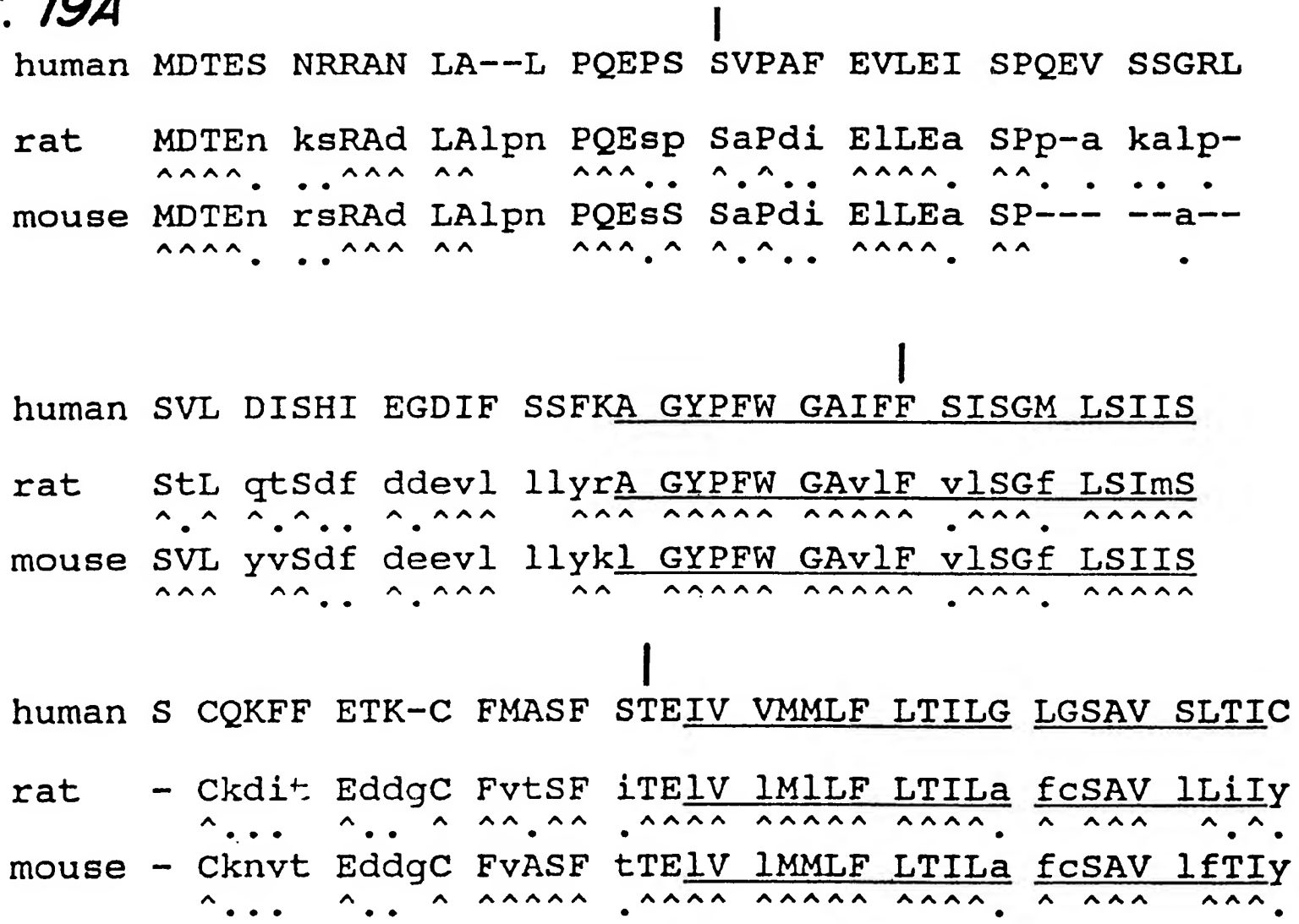

human MDTES NRRAN LA--L PQEPS SVPAF EVLEI SPQEV SSGRL
rat MDTEn ksRAd LAlpn PQEsp SaPdi ElLEa SPp-a kalp-
mouse MDTEn rsRAd LAlpn PQEsS SaPdi ElLEa SP--- --a--
human SVL DISHI EGDIF SSFKA GYPFW GAIFF SISGM LSIIS
rat StL qtSdf ddevl llyrA GYPFW GAvlF vlSGf LSImS
mouse SVL yvSdf deevl llykl GYPFW GAvlF vlSGf LSIIS
human S CQKFF ETK-C FMASF STEIV VMMLF LTILG LGSAV SLTIC
rat - Ckdit EddgC FvtSF iTElV lMlLF LTILa fcSAV lLiIy
mouse - Cknvt EddgC FvASF tTElV lMMLF LTILa fcSAV lfTIy

FIG. 19B

```
                                                  |
LKSAS SPPLH TWLTV LKKEQ EFLGV TQILT AMICL CFGTV VC

eKpAS pPPqq TWqsf LKKEl EFLGV TQvLv qlICL CFGTV VC
 ^.^^ .^^ ^ ^^ .. ^^^^  ^^^^^ ^^^^. .^^^^ ^^^^^ ^^
-K-A- aPPkq TWrTf LKKEl EFLGa TQILv qlICL CFGTi VC
 ^ ^  .^^ ^ ^^ ^. ^^^^  ^^^^. ^^^^. .^^^^ ^^^^^ ^^

          |
ERRNA TYLVR GSLGA NTASS IAGGT GITIL IINLK KSLAY IHIH

ERkNt lYLVR GSLGA NivSS IAaGl GIaIL IlNLs nnsAY mn-y
^^^^.  ^^^^ ^^^^^ ^..^^ ^^.^  ^^.^^ ^^^^. .. ^^ ^^ .
ERkNt lYLVR GSLGA NivSS IAaGT GIamL IlNLt nnfAY mn-n
^^^^.  ^^^^ ^^^^^ ^..^^ ^^.^^ ^^.^^ ^^^^. ..^^^ ^^ ^

                |
   GAGEE LKGNK VPEDR VYEEL NIYSA TYSEL EDPGE MSPPI DL

   riGqE fersK VPdDR lYEEL hvYSp iYSaL EDtrE aSaPv vs
    .^^^ ^. .^ ^^^^^ ^^^^^ ^^^^. .^^.^ ^^. ^ .^.^^
   riGqE LeskK VPdDR lYEEL NvYSp iYSEL EDkGE tSsPv Ds
    .^^^ ^...^ ^^^^^ ^^^^^ ^^^^. .^^^^ ^^.^^ .^.^^ ^
```

FIG. 20

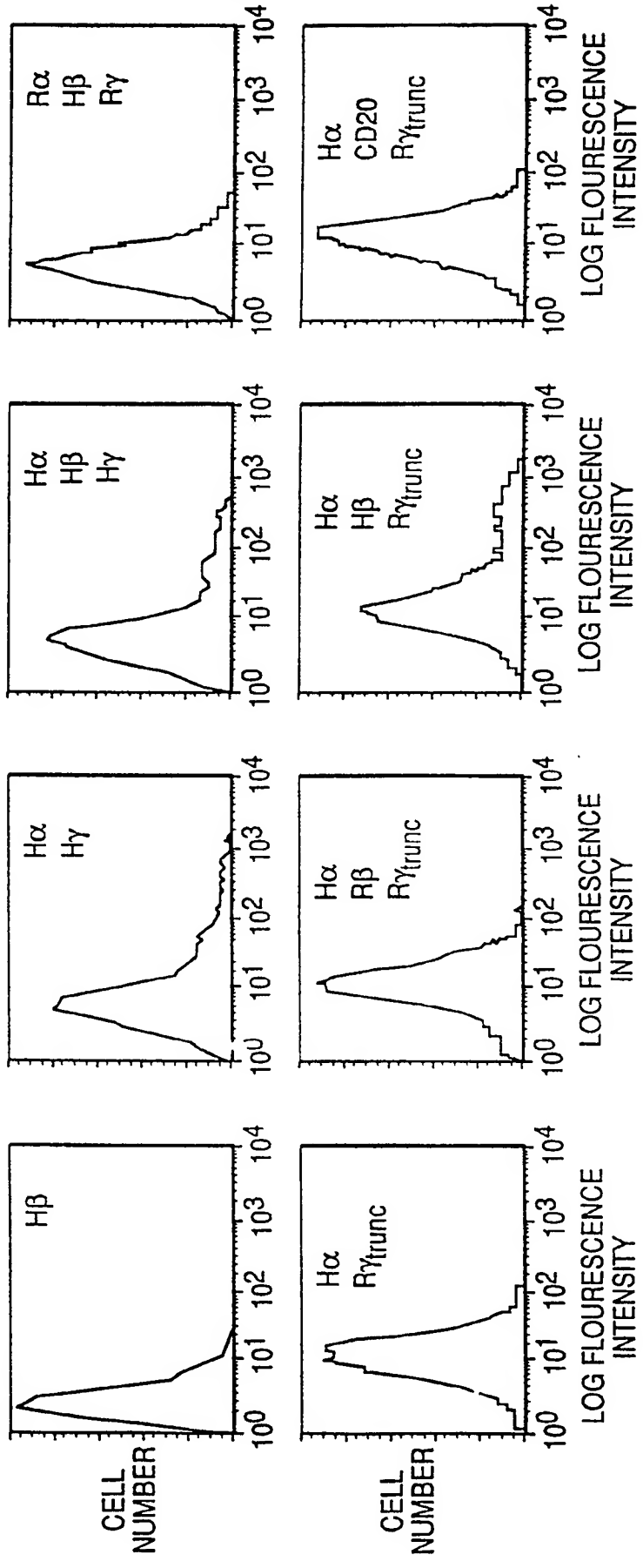
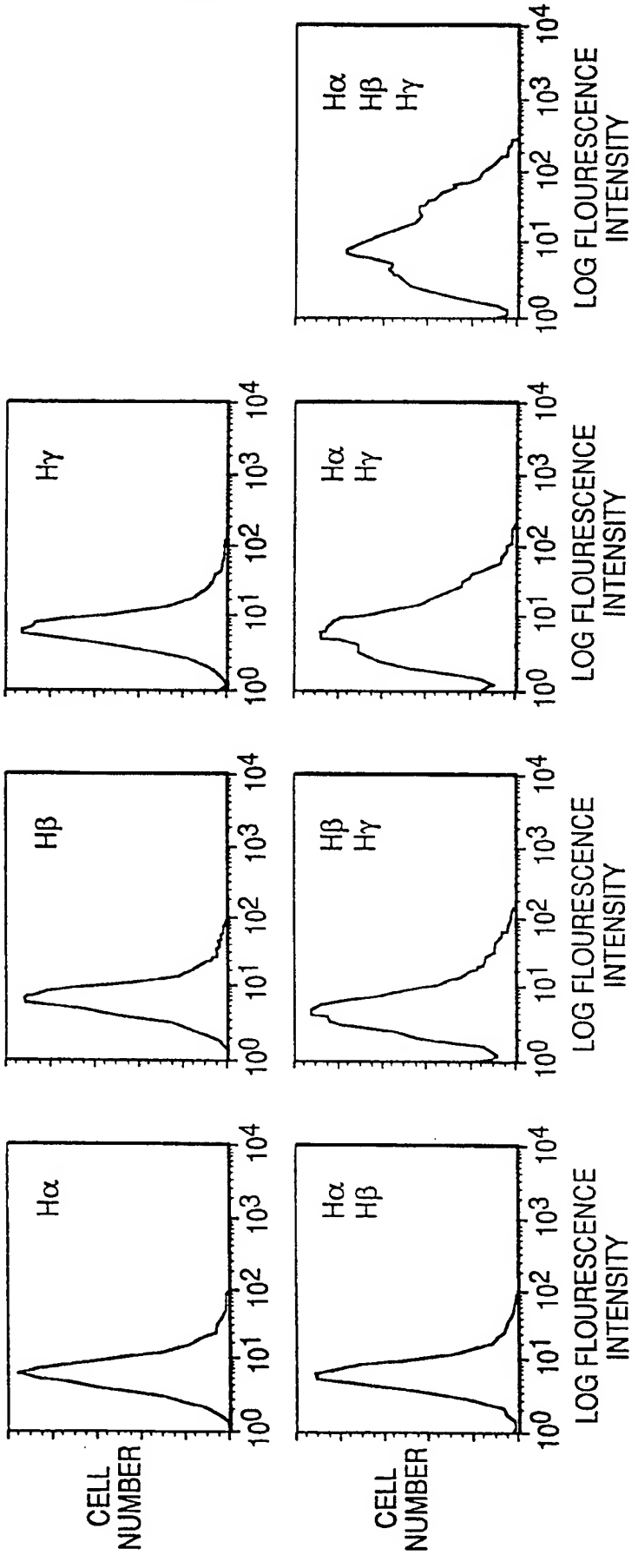

Hβ
Hα
Hγ
Hα
Hβ
Hγ
Rα
Hβ
Rγ
Hα
Rγtrunc
Hα
Rβ
Rγtrunc
Hα
Hβ
Rγtrunc
Hα
CD20
Rγtrunc
CELL NUMBER
LOG FLOURESCENCE INTENSITY
10^0 10^1 10^2 10^3 10^4

FIG. 21


Hα
Hβ
Hγ
Hα Hβ
Hα Hγ
Hβ Hγ
Hα Hβ Hγ
CELL NUMBER
LOG FLOURESCENCE INTENSITY
10^0 10^1 10^2 10^3 10^4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03419

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C07K15/00; C12N5/10; C12P21/00
C12P21/08; A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	DDBJ DATABASE ENTRY HSIGERB, ACCESSION NO. D10583; 25 February 1992, K. MAEKAWA ET AL.; 'Determination of the sequence coding for the beta subunit ofthe human high-affinity IgE receptor' *abstract* & FEBS LETTERS vol. 302, 1992, pages 161 - 165 --- -/--	1-27,29

[10] Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26 AUGUST 1993	23 -09- 1993

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	YEATS S.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA vol. 85, 1988, pages 6483 - 6487 J.-P. KINET ET AL.; 'Isolation and characterization of cDNAs coding for the beta subunit of the high-affinity receptor for immunoglobulin E' cited in the application *abstract; page 6484, left-hand column, second full paragraph; page 6485*	14,15
Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY vol. 265, 1990, pages 6448 - 6452 H. KÜSTER ET AL.; 'Characterization and expression of the gene for the human Fc receptor gamma subunit' cited in the application *abstract; introduction; Table 1*	11,12,23
Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY vol. 266, 1991, pages 22613 - 22620 G. ALBER ET AL.; 'Structure-function relationships in the mast cell high affinity receptor for IgE' *abstract; results*	11,12,23
Y	WO,A,9 004 640 (THE UNITED STATES OF AMERICA) 3 May 1990 *page 8, line 26 - page 12, line 27; page 17, line 10 - page 19, line 1; claims*	11,12,23
P,X	THE JOURNAL OF BIOLOGICAL CHEMISTRY vol. 267, 1992, pages 12782 - 12787 H. KÜSTER ET AL.; 'The gene and cDNA for the human high affinity immunoglobulin E receptor beta chain and expression of the complete human receptor' *whole document*	1-27,29
P,X	FEBS LETTERS vol. 302, 1992, pages 161 - 165 K. MAEKAWA ET AL.; 'Determination of the sequence coding for the beta subunit of the human high-affinity IgE receptor' *abstract; Figure 4*	1-27,29

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9303419
SA 73566

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 26/08/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9004640	03-05-90	AU-B-	632513	07-01-93
		AU-A-	4499589	14-05-90
		CA-A-	2000878	18-04-90
		EP-A-	0439535	07-08-91
		JP-T-	3504439	03-10-91